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Journal of the Fisheries Research Board of Canada

Matière organique dissoute dans l'estuaire maritime du St-Laurent — comparaison et choix des méthodes

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Cauchois D., et M. Khalil. 1974. Matière organique dissoute dans l'estuaire maritime du St-Laurent — comparaison et choix des méthodes. J. Fish. Res. Board Can. 31: 133–139.

Une étude comparative de différentes méthodes d'extraction a été faite en vue d'analyser quantitativement et qualitativement la matière organique présente dans les eaux de l'estuaire maritime du Saint-Laurent. A la suite de quoi on propose deux méthodes d'extraction, l'une par solvant et l'autre par adsorption sur résine (Amberlite XAD-2). Le spectre des produits organiques extraits varie selon la méthode d'extraction. Les matières ainsi extraites sont constituées essentiellement d'hydrocarbures, à une concentration moyenne de 3 à 5 mg/litre et ne représentent que 20% du carbone organique total. On dose également le chlore organique dont la concentration moyenne est de l'ordre de 80 ppb.

Cauchois, D., et M. Khalil. 1974. Matière organique dissoute dans l'estuaire maritime du St-Laurent — comparaison et choix des méthodes. J. Fish. Res. Board Can. 31: 133–139.

To study quantitatively and qualitatively the dissolved organic matter in the St. Lawrence maritime estuary, a comparison between the different extracting methods has been undertaken. We propose two of them; one based on liquid–liquid extraction, the second by adsorption on a resin (Amberlite XAD-2). The methods used remove different spectrums of organic compounds from sea water. Hydrocarbons were found to constitute the major part of the extracts varying between 3 and 5 mg/liter. None of the methods used extracts more than 20% of the total organic matter present. The concentration of the organic chloro-compounds in the water column is in the order of 80 ppb.

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L'ESTUAIRE du St-Laurent est la surface d'eau comprise entre Pointe-des-Monts et la ville de Québec (Forrester 1967). Par ailleurs, l'estuaire est divisé en trois parties (Brunel 1970): l'estuaire maritime qui va de Pointe-des-Monts à l'embouchure du Saguenay, l'estuaire moyen situé entre le Saguenay et l'Ile d'Orléans et l'estuaire fluvial (haut estuaire) qui remonte jusqu'au lac St-Pierre. L'estuaire maritime est donc le site d'une zone de brassage entre les eaux douces du Fleuve et du Saguenay d'une part et les eaux atlantiques en provenance du golfe d'autre part. En outre, le réseau hydrographique du St-Laurent draine à partir des Grands Lacs, des eaux provenant de zones d'exploitation forestière et agricole et de régions hautement industrialisées et par conséquent le fleuve transporte des quantités de matières polluantes. L'estuaire maritime est le site d'une intense activité biologique (Steven 1971) et l'impact de cet apport de matière organique allogène est vraisemblablement important; cependant, très peu de travaux chimiques y ont été effectués (Risi 1932; Nadeau 1938).

Avant d'entreprendre une étude plus précise de la répartition spatio-temporelle de la matière organique dans l'estuaire maritime, nous avons fait

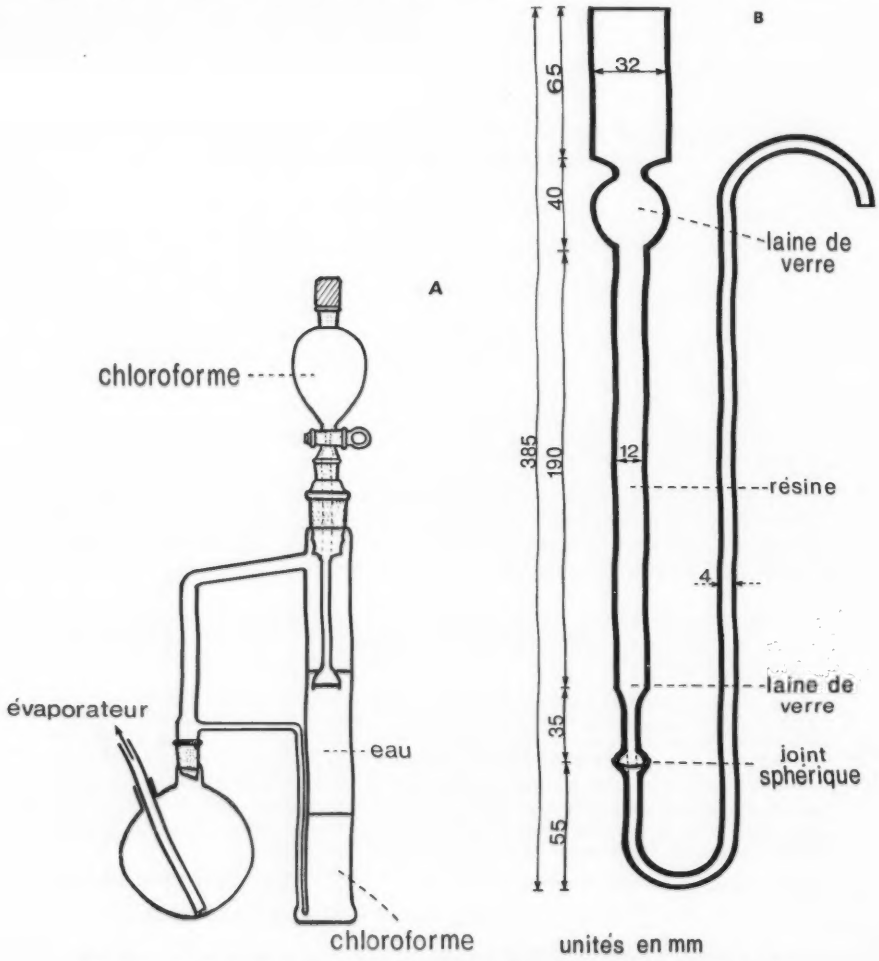

FIG. 1. A, Extracteur Quickfit EX 10; B, colonne d'extraction Méthode C.

une étude plus globale par un échantillonnage représentatif de la zone étudiée en utilisant des stations choisies (Anon. 1972). Afin de procéder à une analyse quantitative et autant que possible qualitative de la matière organique présente, nous avons utilisé des méthodes d'extraction. Essentiellement, deux procédés sont utilisables: l'extraction par solvant et l'extraction par adsorption. Nous avons entrepris de normaliser nos méthodes pour les rendre reproductibles, ce qui nous a également permis de les comparer du point de vue de l'efficacité et du rendement horaire. En même temps, on a fait une étude qualitative de la matière extraite ce qui, joint à un dosage du chlore organique, a permis d'en préciser la composition.

Matériel et Méthodes

Echantillonnage

Les échantillons (20–25 litres) sont prélevés dans des stations préétablies, à cinq profondeurs différentes (3, 10, 20, 30 et 50 m) à l'aide de bouteilles Niskin. On filtre sur papier Whatman No. 4 (macrofiltration). On ajoute 1 cm^3/litre HCl. Les échantillons sont conservés dans des récipients de Nalgène et congelés jusqu'au moment de l'utilisation. Si nécessaire, on filtre sur *Millipore 0.45 μ (microfiltration)* avant l'analyse.

Solvants

Le chloroforme est redistillé et stabilisé avec 1% d'éthanol. Lorsqu'on recycle le solvant, il est d'abord lavé à l'eau distillée, séché sur $CaCO_3$ puis redistillé comme précédemment. L'éthanol absolu est utilisé tel quel, il n'est jamais recyclé.

Appareillage et manipulations

On utilise autant que possible le verre et en cas de nécessité seulement, du Nalgène. Le matériel qui est en contact avec les phases organiques est lavé à l'acide chromique.

Méthode A. — L'eau de mer est placée avec un volume égal de chloroforme dans une bouteille à bouchon rodé de 4 litres. Le tout est agité mécaniquement pendant 20 min et transféré dans une ampoule à décanter. La phase organique est séchée puis évaporée au moyen d'un évaporateur rotatif dont la température est maintenue à 40 C. Lorsque le volume a été réduit à environ 10 cm^3, l'échantillon est transvasé dans un ballon de 50 cm^3. On achève d'évaporer puis on maintient sous vide pendant 4 h.

Méthode B — B_1: l'appareillage est un extracteur Quickfit EX 11. On y place 15 litres de solvant et 5 litres d'eau de mer. L'appareil a été équipé de façon à effectuer l'opération entièrement sous azote. L'extraction est conduite pendant 36 h (pour un rapport solvant/eau de l'ordre de 20). On distille l'excès de chloroforme. Lorsque le résidu est de l'ordre d'un litre, il est soutiré dans l'évaporateur puis traité comme dans la méthode précédente. (Les risques de décomposition peuvent être diminués en remplaçant le chloroforme par le chlorure de méthylène).

B_2 (Fig. 1A): l'appareillage est un extracteur Quickfit EX 10. On y place 25 litres d'eau de mer. Le solvant, (environ 15 litres) provenant d'un réservoir placé au-dessus, passe à travers un diffuseur en verre fritté, il est recueilli dans un ballon, d'où il est soutiré dans un évaporateur, maintenu à 40 C. Lorsque l'extraction est terminée (environ 3 h), l'échantillon est traité comme précédemment.

Méthode C — On prépare une colonne aux dimensions indiquées dans la Fig. 1B remplie d'Amberlite XAD-2. Cette colonne est lavée avec 2 litres d'eau distillée puis 300 cm^3 d'éthanol. Elle est ainsi prête à être utilisée. Le col de cygne est alors monté et l'on fait passer 500 cm^3 d'eau bidistillée (pH = 2) puis 2 litres d'eau de mer à pH 2 (environ 10 h). Le col de cygne est disconnecté, on sèche la colonne avec un courant d'azote durant 4 h puis 12 h dans un dessicateur en présence de KOH et P_2O_5. On élue par 250 cm^3 d'éthanol. L'alcool est évaporé à 40 C. L'échantillon est alors traité comme dans les méthodes précédentes.

Dosage du chlore

Réactifs — Indicateur: solution de 1% de diphenylcarbazone et de 1% de bleu de bromophénol dans l'éthanol. $Hg(NO_3)_2$: entre 10^{-3} et 10^{-4} M dans une solution 10^{-3} N HNO_3 (se conserve à l'abri de la lumière et on la renouvelle tous les 15 jours). NaCl entre 10^{-3} et 10^{-4} M. $Ba(NO_3)_2$ à 1% en solution aqueuse, HNO_3 à 10%. Solution de 2,4 dichlorophénol dans l'isopropanol 10^{-3} à 10^{-4} M (ne pas conserver plus d'une semaine). Purification de l'isopropanol: on ajoute 5 g de sodium à 1 litre d'isopropanol, on porte à reflux pendant 2 h puis on ajoute, avec précaution, 50 cm^3 de H_2O_2 à 50%. On porte de nouveau à reflux pendant 1 h, puis on distille. Tout l'équipement est lavé à l'acide chromique et rincé à l'eau distillée. La solution de $Hg(NO_3)_2$ est standardisée par NaCl comme suit: le dosage est fait sur 2 cm^3, 5 cm^3 et puis 10 cm^3 de la solution de NaCl, on ajoute 15 cm^3 d'isopropanol et 1 cm^3 de $Ba(NO_3)_2$; quatre à cinq gouttes d'indicateur et quelques gouttes de HNO_3 pour avoir une coloration jaune, on dose tout en agitant constamment par $Hg(NO_3)_2$ jusqu'à l'obtention d'une coloration rose violacée.[1]

Le dosage des échantillons

On ajoute à l'échantillon 10 cm^3 d'isopropanol et l'on agite pendant 1 h. On en prélève 5 cm^3 (les 5 cm^3

[1]Le complexe diphenylcarbazone-mercure, de coloration violette, n'est pas soluble dans l'eau; il faut donc s'assurer que le mélange à doser contienne au moins 50% d'isopropanol.

restant servent à doser le chlore minéral) que l'on place dans un ballon avec 10 cm³ d'isopropanol et environ 70 mg de sodium fraîchement coupé. Après 30 min, on porte à reflux pendant 3 h, à l'aide d'un bain d'huile à 110 C. On ajoute alors 1 cm³ de H_2O_2 à 50%, on porte à reflux 1 h et on laisse refroidir. On ajoute 4 cm³ d'eau distillée et 1 cm³ de $Ba(NO_3)_2$ et on dose par $Hg(NO_3)_2$ comme précédemment.[2,3]

Analyse qualitative

Des échantillons d'une même station obtenus par extraction en continu, sont regroupés de façon à avoir une masse d'au moins 100 mg; puis adsorbé sur 3 g de florisil. On fait alors une colonne avec 7 g de florisil que l'on complète avec les 3 g contenant la matière adsorbée. On élue successivement par 100 cm³ d'heptane, de benzène, d'acétate d'éthyle et 20% de méthanol dans l'acétate d'éthyle. Les fractions sont évaporées et pesées. Les spectres I.R. sont pris en film avec un appareil SP 200 G Pye Unicam. Les spectres RMN sont pris en solution dans le CCl_4 sur un appareil R 12 A Perkin Elmer. Les chromatographies en phase gazeuse ont été effectuées avec un appareil Microtech.

[2]La minéralisation du chlore doit s'effectuer entièrement sous azote. Il nous a paru préférable de doser le standard de 2,4 dichlorophénol plutôt qu'un blanc pour tenier lieu de témoin. Le dosage du chlore minéral s'effectue comme indiqué précédemment.

[3]Si la concentration du chlore minéral est supérieure à 10% du chlore total, l'échantillon doit être éliminé.

Tableau 1. Quantité de matière extraite dépendant de la méthode. Erreur maximale estimée 0.5 mg/litre.

Méthode	Type de filtration	Valeur médiane (*mg/litre*)	Nombre d'échantillons traités
A	micro	1	15
A	macro	4	8
B_1	micro	5	10
B_2	micro	6	4
B_2	macro	5	56
C	micro	2	15
C	macro	3	13
Carbone organique total COT	micro	23±1	5

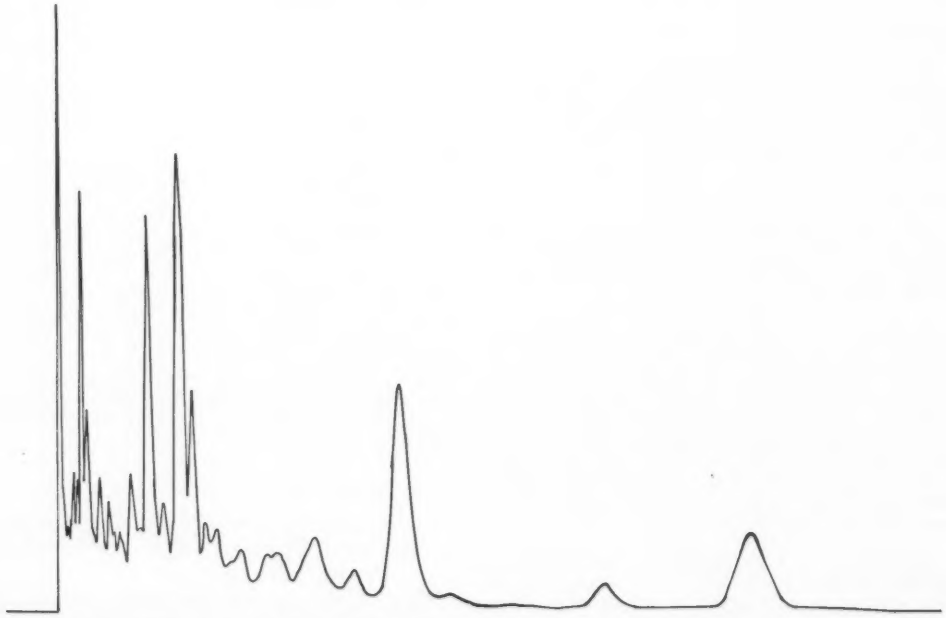

Fig. 2. Chromatogramme sur MT 220 avec capture d'électrons. Température de 170 a 185 C programmée a 4 C/min. Vitesse du gaz vecteur à 80 cm³/min.

MT 220 en utilisant une colonne OV_{17}-QF_1 sur Supelcoport. L'isolation de l'anthracène a été faite sur couche mince de gel de silice en utilisant du CCl_4 comme éluant. Les phthalates ont été mis en évidence par I.R. dans des fractions purifiées par codistillation à l'aide d'un appareil "Sweep codistillator" Kontes. Les spectres de masse ont été effectués par les laboratoires Morgan Schaeffer. L'analyse du carbone organique total (COT) est déterminée au moyen d'un appareil Beckman, modèle 915.

Precision des mesures

En l'absence d'études systématiques, l'estimation de la précision est délicate. Cependant, ayant cherché à déterminer sur quelques échantillons la reproductibilité de l'extraction par solvant ou par résine, on peut évaluer la précision à 0.5 mg/litre en ce qui concerne la quantité de matière extraite. Cette valeur nous semble être l'erreur maximale.

En ce qui concerne le dosage du chlore, le taux de minéralisation a été déterminé pour les produits suivants:

Tableau 2. Concentration en chlore organique total (en ppb) dépendant de la méthode d'extraction. Marge d'erreur estimée à 15%.

Méthode	Type de filtration	Valeur médiane	Nombre d'échantillons traités
A et B_1	micro	80	25
B_2	macro	120	50
C	micro	50	15
C	macro	80	13

95% et plus	chlorure de butyle; chlorobenzène; acide 2,4 dichlorophenylacetique,
90%	hydrate de chloral; 2,4 dichlorophénol,
85%	tetrachloroéthylène,

et puisqu'il s'agit d'un dosage volumétrique, on peut estimer la précision à 15%, ceci dans la mesure où l'échantillon ne développe pas de couleur parasite, ce qui est rarement le cas.

Résultats et Discussion

Les extractions par solvant organique ont été employées par Blumer (1970) qui utilisent le pentane afin d'analyser les hydrocarbures aliphatiques, Levy et Walton (1973) qui utilise le tetrachlorure de carbone pour l'analyse de résidus pétroliers et Barbier et Copin (1970) qui utilisent le chloroforme en vue d'une analyse qualitative et enfin Goldberg et al. (1973) qui utilisent differents solvents organiques. Les extractions par adsorption se font à l'aide de charbon actif ou de résine. Nous avons d'emblée éliminé le charbon actif d'après les travaux de Grigoropoulos et Smith (1971) qui soulignent les difficultés de manipulation et le manque de reproductibilité pour choisir la résine Amberlite XAD-2 (Riley et Taylor 1969; Burnham et al. 1972; Osterroht 1972).

L'influence de la filtration sur l'efficacité de la méthode a été envisagée. Pour cela, des échantillons d'eau de mer sont filtrés sur 0.45 μ Millipore (microfiltration) pendant que d'autres sont filtrés sur papier Whatman No. 4 (Macrofiltration). Les trois méthodes d'extraction ont été étudiées successivement et les résultats obtenus sont regroupés dans le Tableau 1. On notera d'abord que

Tableau 3. Aspects qualitatifs des extraits. Les hydrocarbures sont élués en premier suivi par les organochlorés.

Fraction	% de l'extrait	I.R.	R.M.N.	Chromatographie en phase vapeur
Heptane	30–40%	hydrocarbures saturés	hydrocarbures ramifiés	–
Benzène	30–50%	indication d'aromatiques et phthalates	hydrocarbures ramifiés	voir Fig. 2
Acétate d'éthyle	10–20%	–	–	Voir Fig. 2
Méthanol	<10%	–	–	–

les rendements, par rapport au COT, se situent aux environs de 20%. Cette valeur est en accord avec celles de Barbier et Copin (1970) et d'Osterroht (1972).

Le choix de la méthode se fera donc sur des critères pratiques.

La méthode A a été éliminée car elle nécessite de nombreux transvasements, ce qui augmente notablement les risques de contamination.

La méthode B donne le meilleur coefficient d'extraction. Cependant, elle nécessite un certain appareillage et de gros volumes de solvant, ce qui la rend peu apte à des extractions multiples bien que la variante B_2 élimine les risques de décomposition et permette de traiter quelques échantillons par jour.

Finalement, la méthode C permet de traiter simultanément un grand nombre d'échantillons. Elle nécessite en outre de faibles volumes de solvants et bien qu'elle n'ait pas la meilleure efficacité, nous la préférerons comme méthode de dosage.

Si l'on examine ensuite l'influence de la filtration sur les résultats obtenus, on notera que la méthode B est la moins affectée tout en étant celle qui a le meilleur coefficient d'extraction. Ce fait semble indiquer que la fraction la plus facilement extraite que l'on peut appeler hydrophobe, se trouve en partie adsorbée sur la matière particulée.

Parmi les composés hydrophobes, les dérivés organochlorés (insecticides chlorés, PCB, etc.) semblent être ceux ayant la plus grande incidence écologique. C'est pourquoi nous avons entrepris de les doser.

La spectrométrie de masse de l'un des échantillons a montré la présence de penta et hexa-chlorobiphényles. Par chromatographie en phase vapeur, l'identification d'aucun pesticide chloré usuel n'a pu être faite parmi les nombreux pics observés sur le chromatogramme (Fig. 2). Ceci accentue l'intérêt d'un dosage du chlore organique total. Le dosage est effectué en deux étapes: une minéralisation du chlore organique, par le sodium, suivie d'un titrage par le nitrate mercurique (Anon. 1973). Le taux de minéralisation a été mesuré à l'aide de différents composés et trouvé supérieur ou égal à 90%. Cette analyse du chlore organique a été effectuée sur des échantillons extraits par les méthodes précédemment décrites et les résultats sont rassemblés dans le Tableau 2.

La première remarque que l'on peut faire c'est que, si les valeurs médianes sont d'un ordre de grandeur comparable, quelle que soit la méthode, les valeurs extrêmes sont 10 fois plus fortes dans les méthodes d'extraction par solvant que dans la méthode par résine. Ceci semble indiquer que certains échantillons subissent une contamination de la part du solvant. On notera, de plus, que la valeur du chlore organique total dosé après une macrofiltration est supérieure. Ceci confirme l'hypothèse déjà émise de l'adsorption de la matière hydrophobe sur les particules en suspension.

Les composés organochlorés ne représentant que 1–5% de la matière extraite, il était intéressant d'en préciser sa composition.

Les résultats des études qualitatives sont regroupés dans le Tableau 3 et l'on note que 70 à 80% de l'extrait est constitué d'hydrocarbures ou tout au moins de composés très faiblement fonctionnalisés. Les spectres I.R. et R.M.N. sont en effet typiques d'hydrocarbures saturés, ramifiés. La spectrométrie de masse indique en outre que les chaînes en C_{25} sont les plus abondantes. Des spectres I.R. de fractions plus purifiées révèlent la présence de composés aromatiques et d'esters lourds dissous dans ces hydrocarbures. Ceci est confirmé par l'isolation d'anthracène pur et par la présence de pics caractéristiques de phthalates de butyles et d'octyles obtenus par spectrométrie de masse. La présence des phthalates a par ailleurs été signalée dans l'eau de mer (Barbier et Copin 1970; Mayer et al. 1972).

Tout ceci montre que les méthodes d'extraction ne peuvent être une technique de mesure de la matière organique totale mais seulement de la fraction hydrophobe qui est essentiellement composée d'hydrocarbures. Quant au dosage du chlore organique, nous préférons l'extraction par résine précédée d'une macrofiltration.

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Nutrient Composition of Experimentally Produced Meals from Whole Argentine, Capelin, Sand Lance, and from Flounder and Redfish Filleting Scrap

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Nutrient composition studies have been carried out on single samples of meals produced from whole Atlantic argentines (*Argentina silus*), capelin (*Mallotus villosus*), American sand lance (*Ammodytes americanus*) and from filleting scrap from flounders (Pleuronectidae sp.) and redfish (*Sebastes marinus*).

Analyses included protein, fat, ash, moisture, minerals, vitamins, available lysine, amino acids, and protein digestibility. The whole fish meals appeared nutritionally equal to good herring meal. The filleting scrap meals were lower in protein content but the nutritional values of the proteins were equivalent to that of herring.

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Nous avons déterminé les substances nutritives d'échantillons uniques de farines préparées à partir de grandes argentines (*Argentina silus*), de capelans (*Mallotus villosus*) et de lançons d'Amérique (*Ammodytes americanus*) entiers, et de déchets de découpage en filets de plies (Pleuronectidae sp.) et de sébastes atlantiques (*Sebastes marinus*).

Nous y avons mesuré les protéines, graisses, cendres, humidité, minéraux, vitamines, lysine disponible, aminoacides et digestibilité des protéines. Les farines de poissons entiers semblent avoir une valeur nutritive égale à une bonne farine de hareng. Les farines préparées à partir de déchets de filets ont une teneur en protéines plus faible, mais la valeur nutritive des protéines équivaut à celle du hareng.

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THE chief raw materials for fish meal production on the East Coast of Canada are herring and scrap and cuttings (offal) from filleting plants. The catch of herring reached a peak in 1968, but has been declining each year since. The plants that were built especially for herring reduction are large with a high daily production capacity and very few of these are utilized for other raw materials in the off season (Jangaard 1967). A number of species have been mentioned as being present in

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sufficient quantities to warrant a new fishery enabling these plants to better utilize their production capacity.

Capelin (*Mallotus villosus*) occur in large quantities off the coasts of Newfoundland and Labrador and have potential for meal production (Devold et al. 1971; Dragesund and Monstad 1972; Winters 1970). Similarly, American sand lance (*Ammodytes americanus*) and Atlantic argentines (*Argentina silus*) occur in sufficient numbers to be considered as alternate raw materials (Leim and Scott 1966; Jones 1968; Cadegan 1971; Scott 1969 and 1971; Emery and McCracken 1966).

The landings of redfish (*Sebastes marinus*) and flounder (Pleuronectidae sp.) have been increasing. Although only the scrap or cuttings of these fish are used for meal they are becoming important raw materials.

Since many commercial meals result from the mixing of several species there appeared to be a need for analytical results from meals of known species composition. Studies on the nutrient composition of fish meals from the Atlantic coast of Canada have been carried out earlier on herring meals (Power et al. 1969), and whitefish meals (Regier et al. 1973) by this laboratory. The study reported here describes the nutrient content of single samples of meals experimentally produced from species currently considered to be underutilized and from filleting scrap from flounders and redfish (ocean perch).

Experimental

Sand lance were caught on the banks off the Nova Scotian coast. One catch of about 45.4 t (50 tons) was commercially processed into meal and oil and we obtained a representative sample for study.

The capelin were caught off Newfoundland, the argentines on the banks off Nova Scotia, and the redfish and flounder filleting scrap were obtained from a filleting plant. All samples were frozen and shipped to the Vancouver Laboratory of the Fisheries and Marine Service for processing.

The meal samples were prepared in a fish meal pilot plant having a capacity of about 23 kg (50 lb) of fish per hour (Claggett 1968). The operational data are

TABLE 1. Processing data for fish meal pilot plant.

			Filleting scrap	
	Capelin	Argentine	Redfish	Flounder
Wt of raw material processed (*kg*)	220	122	218	190
Meal recovered (*kg*)	34	20	32	29
Yield of presscake meal (%)	15.4	16.4	15.0	15.5
Oil recovered (*kg*)	3.6	3.2	6.1	4.1
Yield of oil (%)	1.7	2.6	2.8	2.1
Press liquor (stickwater)				
Vol (*liters*)	72	40	135	117
Oil content (%)	4.8	8.1	5	4
Solids content (%)	14	12	9.1	8.4

TABLE 2. Proximate analyses of fish meals (%).

	Sand lance		Capelin		Argentine		Redfish filleting scrap		Flounder filleting scrap	
	1[a]	2[b]	1	2	1	2	1	2	1	2
Moisture	2.3	8.0	13.6	8.0	6.4	8.0	1.2	8.0	6.3	8.0
Ether extract (fat)	8.8	8.3	8.8	9.3	8.6	8.5	13.0	12.2	10.2	10.0
Protein	78.7	74.4	68.4	72.2	76.1	75.0	60.0	56.2	59.9	58.9
Ash	11.5	10.9	8.3	8.8	9.2	9.1	25.4	23.8	23.2	22.8

[a]Meal as received for analysis.
[b]Results adjusted to 8% moisture for comparative purposes.

given in Table 1. The solids from the stickwater were not added to the meal and the samples are, therefore, so-called presscake meals rather than whole meals. The samples were partially thawed, ground, and then cooked with indirect steam heating at a temperature of 103–106 C (218–222 F) at 34.5 KPa for 10 min. The temperature of the fish leaving the cooker was about 80 C (175 F). The press was set as tight as possible and the presscake was dried in a steam dryer operated at 110 C (230 F) which gave a meal outlet temperature of 71 C (160 F). No difficulties were encountered in separating the oil from the stickwater.

Chemical analyses were carried out by the WARF Institute Inc., Madison, Wisc. Pepsin digestibility studies, chemical estimations of biologically available lysine, and biological assessment of protein quality were carried out at the University of British Columbia. The analytical methods have been previously described (Regier et al. 1973).

Results and Discussion

Due to the difficulties of "running in" the pilot plant equipment for each sample, the moisture content of the meals was quite variable, and for comparative purposes the analytical results have also been adjusted to a moisture content of 8%.

The proximate composition of the five samples is shown in Table 2. The analyses in subsequent tables are, however, given as part of the meal as it was received.

The data for the meals produced from sand lance, capelin, and argentine indicate that meals with protein contents and nutritional values as high as herring meal can be produced from these species. Comparisons between herring meals and capelin and sand lance meals produced in Norway have been published (Sparre 1971). The average protein content of 19 experimentally produced Norwegian capelin meals was 70.2% with a fat content (ether extract) of 8.8%. The analyses given in Table 2 are similar to these. Since capelin is a rather difficult raw material to handle, special procedures, such as "ageing" the fish, are necessary to liberate the oil so that it can be removed in the press (Jangaard 1973). If this procedure is not followed a high fat meal can result. Other samples of commercial capelin meals analyzed in Norway (Olsen 1967) show protein levels from 65.8 to 70.8%. The meal with the lowest protein value had a high fat content (13.8%). Analytical data from Icelandic commercial capelin meals (Thorbjarnarson 1967) also show consistently high fat levels (13–11%) with the correspondingly low protein contents (65–68%). However, reduction plants, especially modified for capelin reduction and with experienced supervisory personnel, should consistently be able to produce meals with fat levels below 10%.

Sand lance meals produced experimentally in Norway (Opstvedt et al. 1970) had an average protein content of 71.3%, fat 7.7%, and ash 11.8% at a moisture content of 7.8%. Three commercial samples (Olsen 1967) had on an average 72.2, 8.2, 12.0, and 6.7%. Thorbjarnarson (1967) gives the corresponding values for Icelandic commercial sand lance meals as: 70–73% protein, 8.5–11% fat, and 8% moisture. The proximate composition of the experimental Canadian Atlantic sand lance meal is similar to these.

Although argentines have been exploited as a food fish, especially by USSR and Japanese vessels for several years off the coast of Nova Scotia, very little information is available on the composition of argentine meal. Data from Table 2 indicate that

TABLE 3. Vitamin analyses of fish meals — μg/g (ppm).

	Sand lance	Capelin	Argentine	Redfish filleting scrap	Flounder filleting scrap
Thiamine	0.2	1.20	0.3	0.5	0.5
Riboflavin	12.5	7.55	8.30	4.65	11.7
Pantothenic acid	16.1	19.6	15.8	5.32	3.7
Niacin	89.1	54.1	68.1	22.5	27.0
Choline chloride	9580	6100	4440	3500	3050
Biotin	0.364	0.469	0.300	0.213	0.167
Folic acid	0.321	0.408	0.187		
Vitamin B-12	0.177	0.107	0.0597	0.164	0.129

meal from this species has a high protein, low ash, and medium fat content.

The proximate composition of the meals made from filleting scrap and cuttings of redfish and flounder are typical of this type raw material in that the ash content is in the range of 20–25%. The protein content is slightly on the low side and fat somewhat high, indicating some difficulties in processing this raw material.

The vitamin content of the meals is given in Table 3. Except for the capelin meal, the thiamine content was low as compared to whitefish meals but were in the same range as herring meals, (Regier et al. 1973; Power et al. 1969; Karrick et al. 1957). Significantly higher results for niacin, pantothenic acid, riboflavin, and B-12 were reported in Norwegian capelin meal by Sola and Kalhagen (1967) which may be related to the loss of the water solubles in the experimental meal. In general, however, the experimental meals are good vitamin sources when compared with herring meal.

Several of the mineral values (Table 4), especially calcium, phosphorus, boron, chromium, and strontium, are higher in the redfish and flounder meals due to the elevated ash content in these samples. The arsenic content in the flounder meal is much higher than in the other meals, but Lunde (1968a) has shown that there is a considerable variation among fish meals even from the same species. He has also shown (Lunde 1967, 1968b) that organic arsenic compounds can be found in the oil. The selenium contents of the meals are above the average for fish meals found by Kifer et al. (1969) but do not seem excessive when compared to the 2.0 ppm found in whitefish meal samples (Regier et al. 1973).

The amino acid composition is given in Table 5 and values for pepsin digestibility, available lysine, supplementary protein values, and feed conversion are given in Table 6.

The lysine content is generally high in all samples, except the flounder meal, and compares favorably with herring meals (Power et al. 1969). The value found for the flounder meal is still well within the range commonly listed for whitefish and offal meals (Miller 1970). The pepsin digestibility is high in all samples and the available lysine also compares favorably with herring meals. The supplementary

TABLE 4. Mineral analyses of fish meals.

	Sand lance	Capelin	Argentine	Redfish filleting scrap	Flounder filleting scrap
			(%)		
Calcium	1.8	1.9	2.6	8.7	8.8
Magnesium	0.13	0.24	0.24	0.15	0.17
Phosphorus	1.3	1.6	1.9	3.4	3.3
Potassium	1.3	1.1	1.2	1.0	1.0
Sodium	0.92	0.66	0.47	0.48	0.68
Sulfur	0.53	0.67	0.64	0.49	0.65
			µg/g (ppm)		
Aluminum	42	97	110	139	199
Arsenic		2.5	4.9	3.3	22.0
Barium	2.0	2.0	1.6	3.8	3.9
Boron	5.3	7.9	7.9	12.9	17.5
Cadmium		0.34	0.13	0.2	0.2
Chromium	1.5	1.5	2.7	17.4	23.7
Cobalt	0.05	0.10	0.18	0.05	0.05
Copper	2.7	12	11	13.5	14.1
Iodine	28	0.50	0.77	1.54	3.4
Iron	85	575	675	250	662
Lead		13.4	7.4	0.8	2.8
Manganese	4.5	5.8	4.6	6.3	14.2
Mercury		0.10	0.29	0.2	0.2
Molybdenum	0.05	3.8	4.2	8.8	8.4
Selenium		1.7	1.8	2.6	3.0
Strontium	90	100	110	362	416
Zinc	95	142	101	74	82

protein values compare well with herring meal and the values found for the two offal meals are slightly higher than those found for whitefish meals (Regier et al. 1973).

The nutritional quality of meals made from whole sand lance, capelin, and argentine can, therefore, be considered to be equal to herring meal. The meals prepared from filleting scrap from flounder and redfish have a lower protein content, but the nutritional value of the protein in the meal has been demonstrated to be equal to that of herring meal, as assessed by biological tests with chicks.

TABLE 5. Amino acid analyses of fish meals.

	g/100g protein				
	Sand lance	Capelin	Argentine	Redfish filleting scrap	Flounder filleting scrap
Lysine	7.96	8.23	8.42	7.88	6.56
Histidine	2.33	2.50	2.67	2.33	5.98
Arginine	6.34	6.18	6.28	6.74	5.98
Aspartic Acid	9.68	9.01	8.94	9.35	9.42
Threonine	4.37	4.61	4.43	4.32	4.31
Serine	4.14	4.30	3.98	4.56	4.84
Glutamic Acid		13.86	13.15	13.15	13.63
Proline	4.47	3.63	3.35	4.82	5.34
Glycine	7.44	5.25	5.32	7.98	8.26
Alanine	6.16	5.94	5.62	6.90	6.21
Valine	4.98	4.87	4.57	4.75	4.74
Methionine	3.13	3.10	3.08	3.18	2.92
Isoleucine	4.70	4.27	4.30	4.10	4.02
Leucine	7.70	9.66	7.45	7.12	7.24
Tyrosine	3.70	3.67	3.46	3.35	3.21
Phenylalanine	4.03	4.18	4.18	3.98	3.81
Tryptophan	0.97	1.17	1.26	1.00	1.00
Cystine	–	1.32	1.42	1.78	1.42
Ammonia	0.66	0.82	0.80	0.75	0.65

TABLE 6. Pepsin digestibility, available lysine, supplementary protein values, and feed conversion for fish meals.

	Sand lance	Capelin	Argentine	Redfish filleting scrap	Flounder filleting scrap
Pepsin digestibility[a] (% of crude protein)	96.6	94.1	96.6	95.8	93.5
Available lysine (% of crude protein)	6.7	6.2	6.2	6.0	5.9
Supplementary protein[b] values, 4% of protein	124	132	131	123	122
Feed conversion g gain/g feed consumed		0.40	0.40	0.35	0.34
Supplementary protein values, 8% of protein	108	102	103	105	107
Feed conversion g gain/g feed consumed		0.47	0.48	0.46	0.45

[a]Pepsin (activity 1:20,000) at a concentration of 0.02%.
[b]Chicks 4 wk on test.

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The Biology of *Bothrimonus* (=*Diplocotyle*) (Pseudophyllidea: Cestoda): Detailed Morphology and Fine Structure[1]

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BURT, M. D. B., AND I. M. SANDEMAN. 1974. The biology of *Bothrimonus* (=*Diplocotyle*) (Pseudophyllidea:Cestoda): detailed morphology and fine structure. J. Fish. Res. Board Can. 31: 147–153.

Light and electron microscopy were used to describe the functional morphology of *Bothrimonus sturionis* in detail. In particular, the musculature, nervous system, osmoregulatory system, and tegument are dealt with, and the findings compared with those of other workers. The musculature of the scolex consists of several interrelated systems, the structure of each being discussed in relation to its function. Associated with the regular nervous system, considered typical of cestodes, is an extensive system of giant nerve fibers. The osmoregulatory system is unusual in that there are lateral "excretory" pores in many proglottides which open directly to the exterior of the worm. The microtriches of the tegument are long, like those of other primitive cestodes, and are covered by a noncellular sheath while the worm is in its gammarid host. The sheath is lost when the worm becomes established in its fish host; the nature and function of the sheath are discussed.

BURT, M. D. B., AND I. M. SANDEMAN. 1974. The biology of *Bothrimonus* (=*Diplocotyle*) (Pseudophyllidea:Cestoda): detailed morphology and fine structure. J. Fish. Res. Board Can. 31: 147–153.

Nous décrivons en détail, à l'aide de la microscopie optique et électronique, la morphologie fonctionnelle de *Bothrimonus sturionis*. Nous étudions plus particulièrement la musculature, le système nerveux, le système osmorégulateur et le tégument, et comparons nos observations avec celles d'autres chercheurs. La musculature du scolex est constituée de plusieurs systèmes reliés entre eux, la structure de chacun étant examinée en rapport avec sa fonction. Associé au système nerveux régulier, considéré comme typique des cestodes, se trouve un vaste réseau de fibres nerveuses géantes. Le système osmorégulateur a ceci de particulier qu'il renferme des pores "excréteurs" latéraux dans plusieurs proglottis, communiquant directement avec l'extérieur du ver. Les microtriches du tégument sont longs, comme ceux des autres cestodes primitifs, et sont recouverts d'une gaine acellulaire lorsque le ver est dans un gammaride qui lui sert d'hôte. Le ver se débarasse de sa gaine lorsqu'il s'établi dans l'hôte suivant, un poisson. Nous discutons de la nature et de la fonction de la gaine.

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A LARGE number of cestodes, identified as *Bothrimonus sturionis* has been examined from different hosts and from different localities as follows: *Gammarus oceanicus* (Newfoundland, Canada), *Marinogammarus finmarchicus* (Scotland), *M. pirloti* (Scotland), *Apeltes quadracus* (Newfoundland, Canada), *Microgadus tomcod* (Massachussetts, USA), *Oncorhynchus gorbuscha* (Attu Island in the Pacific), *Pseudopleuronectes americanus* (New Brunswick, Canada), *Salmo salar* (Newfoundland, Canada), and *Salvelinus alpinus* (Greenland).

The taxonomic position of *B. sturionis*, with a general description of the worm, its reproductive system, and discussion of the systematics, was published earlier (Burt and Sandeman 1969) as was an account of its ecology, life cycle, and evolution (Sandeman and Burt 1972). The present work presents a detailed description and discussion of the musculature, nervous system, osmoregulatory system, and fine structure.

[1]This is the last of three papers on the biology of *Bothrimonus*. The previous two appeared in this Journal: Vol. 26(4) 1969, and Vol. 29(10) 1972.

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Description

The diagnostic characters of *B. sturionis* can be summarized as follows: length: 8–110 mm; size of scolex: 0.39–1.14 mm deep by 0.31–0.8 mm broad by 0.135–0.68 mm long; two suckers, one dorsal and one ventral, up to 750 μ diameter; no external segmentation; genital apertures normally on ventral surface, in midline; opening of cirrus sac in front of opening of female pore; distance between cirrus sacs usually 600–700 μ; size of cirrus sac: 140–320 μ long by 100–200 μ diameter; everted cirrus: up to 170 μ long by 120–140 μ diameter at base; number of testes variable; diameter of testes 45–100 μ; vitellaria in two continuous, cortical, lateral fields; ovary bilobed, each lobe containing 3–9 lobules; shell gland alternates irregularly to right or left of midline, about 100 μ diameter; uterus prominent when full of eggs; size of eggs 32–45 μ by 21–32 μ; oncosphere hooks: 9–12 μ long.

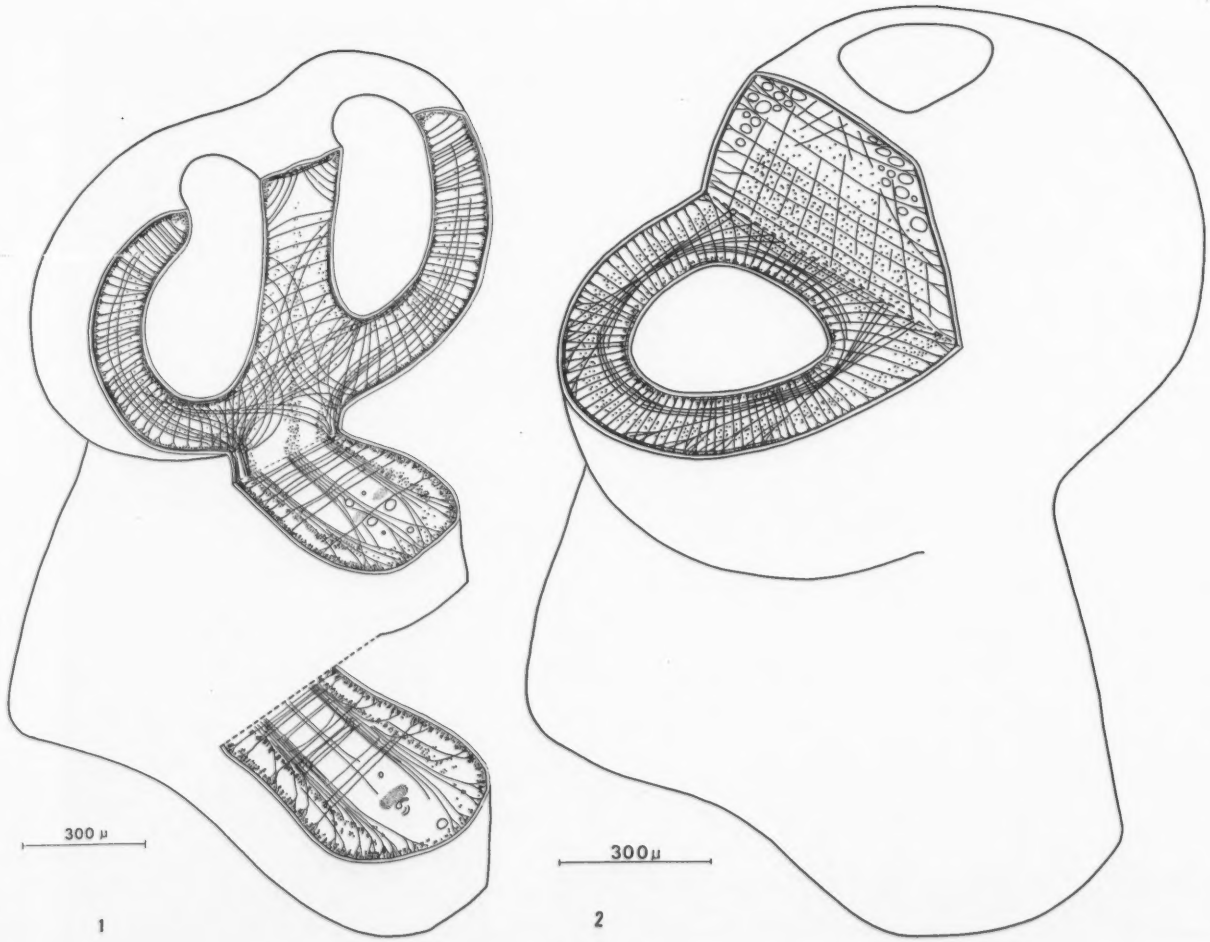
Musculature

In the strobila and neck region (Fig. 1, 2) the following muscle layers are present: immediately under the basal lamella of the tegument and closely associated with it is a thin layer of transverse muscle (Fig. 3), seen best with an electron microscope, under which is a layer of single longitudinal fibers. Beneath these two layers and embedded in the outer half of the cortex are more longitudinal fibers either single or loosely in groups of two or three. These are most numerous in the cortex of the dorsal and ventral surfaces of the worm; few are seen in the lateral edges of the strobila. The cortex is separated from the medulla by an outer layer of longitudinal fibers and an inner layer of transverse fibers. The longitudinal fibers are absent from the two lateral regions and are arranged rather loosely in bundles with up to eight or nine fibers in each bundle. The transverse fibers are not arranged in bundles and a few are found externally to the longitudinal layer. These fibers branch finely at their outer ends and the fine branches are inserted in the fibrous layer (Fig. 3) on the lateral, dorsolateral, and dorsoventral regions of the basal lamella of the tegument. Dorsoventral fibers are also present running through the medulla. The fibers are single and are again finely branched and inserted on the basal lamella on the dorsal and ventral regions. Both the transverse and dorsoventral fibers may be concentrated in certain regions after displacement by some of the large organs of the body such as the uterus.

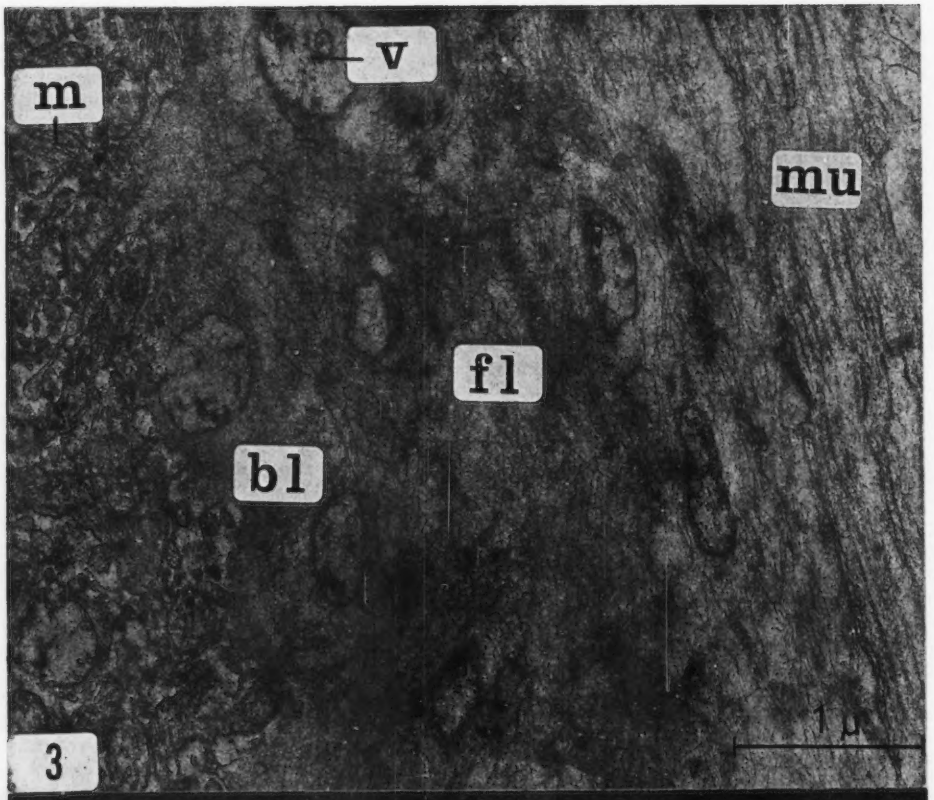
Immediately behind the scolex (Fig. 1) a tract of transverse fibers runs laterally across the center of the body. A few run through the cortex, again finely branched and inserted in the basal lamella at the lateral borders of the body. This tract extends only a short distance into the scolex.

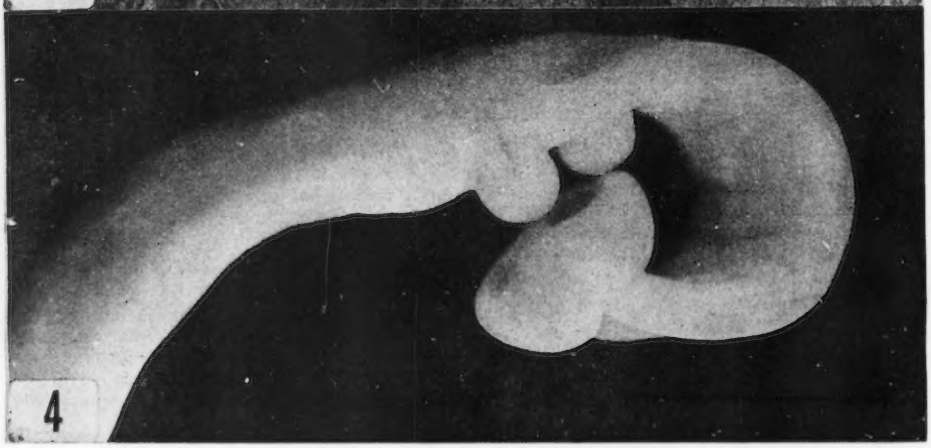
The musculature of the scolex itself (Fig. 1, 2) is dominated by the fibers running between the internal and external wall of each bothrium. In the central region between the two suckers the fibers run from the inner border of one sucker to the inner border of the other. Examination of electron micrographs indicates that the finely branching ends of the radial fibers are continuous with the thin layer immediately under, and closely associated with, the basal lamella of the tegument. Other prominent muscle tracts in the scolex are as follows: 1, a layer of single longitudinal muscles, lying immediately under the basal lamella and thin layer of tegument of the external surface of each bothrium; 2, immediately interior to the layer of longitudinal muscle described above is a thin layer of single, circularly directed fibers; 3, a concentration of fibers immediately under the tegument surrounding the opening of each bothrium and forming a sphincter-like band. These fibers are continuous with the horizontal layers in 1 and 2; 4, in the posterior half of the scolex there are several tracts of muscles running longitudinally. One of these lies inside the thin, outer longitudinal, and horizontal layers. The muscles originate either on the outer posterior surface of the bothria, or on the inner surface of the junction between each bothrium and the neck. They pass down the neck joining the two dorsal and ventral bands of longitudinal fibers in the strobila and a few cross over to the dorsoventral fibers near the base of the scolex: 5, a second but less extensive layer of longitudinal muscle lies just inside the inner surface of each bothrium extending not more than half way up to the opening of the bothrium; 6, between the two longitudinal layers described in 4 and 5 is an extensive tract of horizontal fibers around each bothrium. However, it is not a simple ring of fibers as can be seen in Fig. 2. The anterior extension of the strobila between the two bothria, in addition to having the fibers described so far, possesses: 7, fibers which are inserted on the inner wall of each bothrium, and which pass across and backward to join the dorsal or ventral longitudinal tracts of the neck; 8, radial fibers from the internal wall of the anterior of each bothrium which, instead of crossing to the other bothrium, are inserted on the tip of the interbothrial mass; 9, a few transverse and dorsoventral fibers lying immediately under the tegument on the tip of the interbothrial mass.

The anterior portion of the interbothrial mass is provided with relatively little muscle but contains mainly parenchymal cells, the anterior portion of the nervous system, and a considerable network



FIGURES 1–2
FIG. 1. Musculature of scolex and part of strobila (×42); FIG. 2. Musculature of scolex (×38).





FIGURES 3–4

FIG. 3. Electron micrograph of basal lamella, fibrous layer, and associated muscle (×23,220). bl, basal lamella; fl, fibrous layer; m, mitochondrion; mu, muscle; v, vesicle; FIG. 4. Scolex and part of strobila to show effect of sucking action of bothria (×31).

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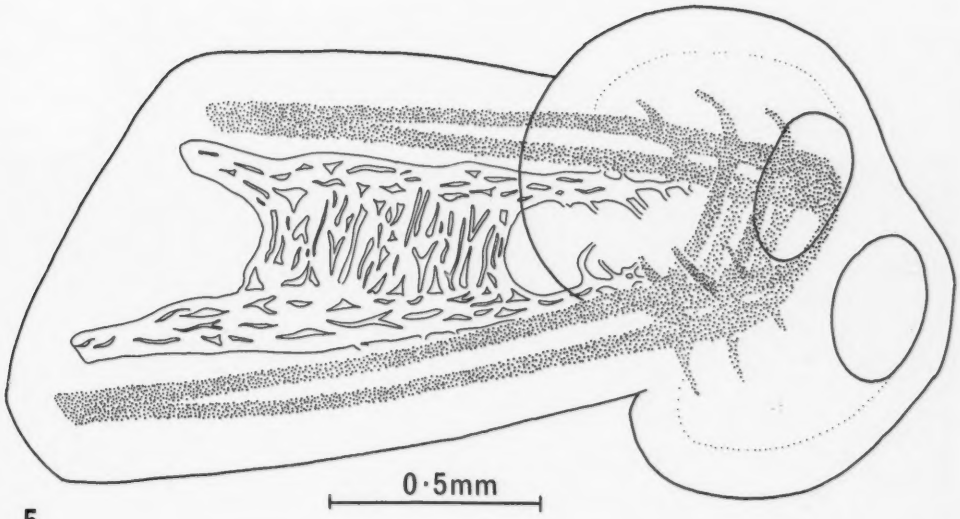

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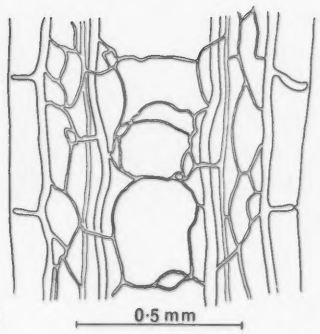
FIG. 5. Nervous systems in scolex and part of strobila (×35).



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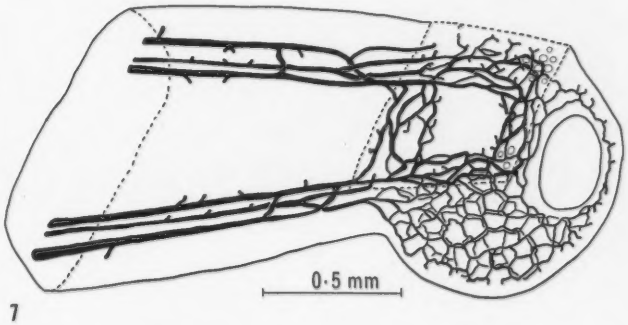

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FIGURES 6–7. Osmo-regulatory system.
FIG. 6. Main vessels in strobila (×42); Fig. 7. Main vessels and reticular system in scolex (×21).

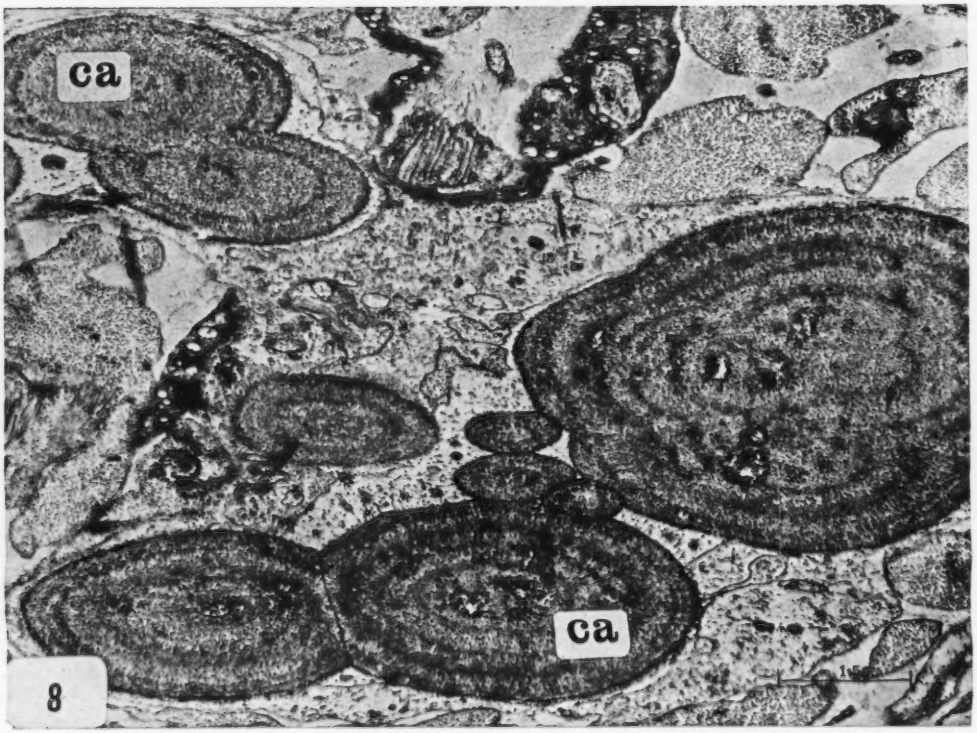
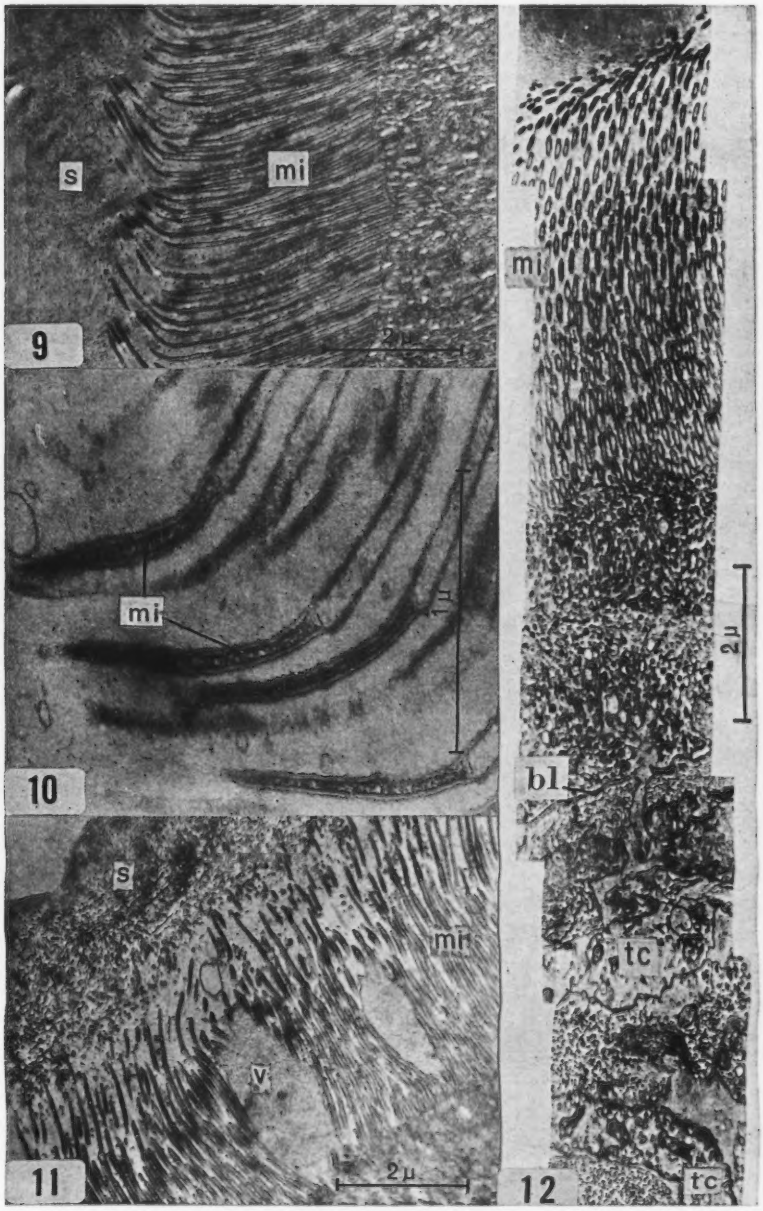


FIG. 8. Electron micrograph of calcareous corpuscles (×9230). ca–calcareous corpuscle.



FIGURES 9–12. Electron micrographs of tegument.

FIG. 9. Strobilar tegument of worm from gammarid (×9240); FIG. 10. Microtriches (×35,420); FIG. 11. Scolex tegument of worm from gammarid (×8470); FIG. 12. Panorama of tegument of worm from fish (×9625). bl–basal lamella; mi–microtriches; s–sheath; tc–tegumental cell; v–vesicle.

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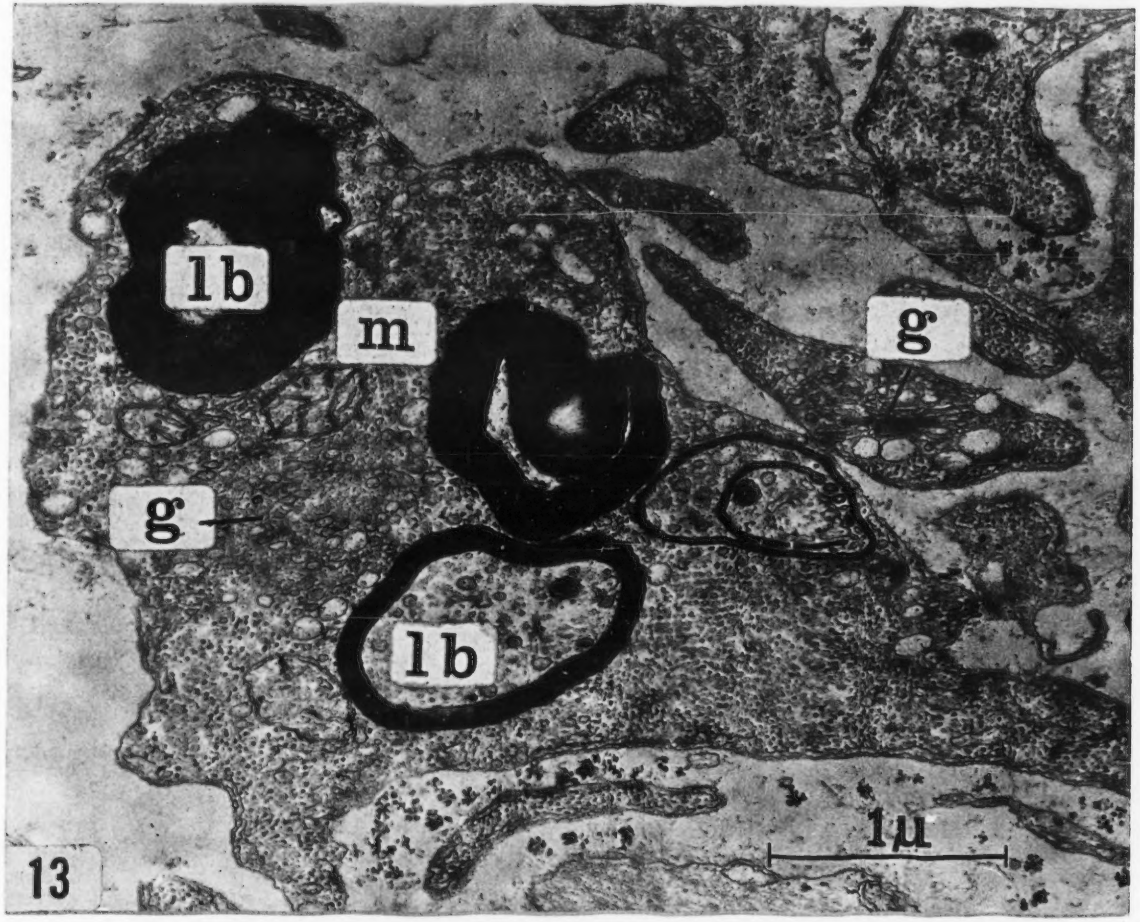

FIG. 13. Electron micrograph of part of tegumental cell with lamellated bodies (×24,000). g-golgi; lb–lamellated body; m-mitochondrion.

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of excretory canals and vesicles. There are few longitudinal fibers except around the periphery.

The scolex functions effectively as an adhesive organ as seen from the number of worms attached firmly to the intestinal wall of our New Brunswick flounder material. The worms attach to the surface presumably by producing a suction or negative pressure in the lumen of the bothrium, which draws the surface in to completely fill the lumen. The result of this action can be seen in Fig. 4—a photograph of a worm that had attached to its own strobila. Contraction of the powerful radial muscles would enlarge the cavity of the bothria and provide the suction or negative pressure required. Presumably the remaining muscles are antagonistic, with the exception of the thin muscle layers immediately under the tegument which give it support. If this is the case then it is doubtful if the sphincter-like band of muscle around the opening of each bothrium is a true sphincter, as it appears to be homologous to the fibers underlying the tegument.

The three genera *Bothrimonus*, *Diplocotyle*, and *Didymobothrium* are separated largely on the shape of the scolex. As was described earlier (Burt and Sandeman 1969: Fig. 1, 2, and 3), however, *Bothrimonus* shows all shapes depending on the degree of contraction of the scolex musculature. In the relaxed state, the scolex takes on an "arrowhead" shape. As the radial muscles contract, increasing the volume of the suckers, expansion in an anterior direction results in the shape previously associated with *Diplocotyle*.

A further increase in the area of the walls of the bothria, derived from more intense contraction of the radial muscles, will force the unattached portions of the bothria even further outwards and forwards. The positions of the circular bands of muscle around the opening of the bothria, relative to the interbothrial mass, restricts the direction in which expansion takes place resulting in the appearance, previously associated with *Bothrimonus*, of a single anterior sucker. Expansion of the lateral borders of the interbothrial mass, to form the lateral edges of the single opening that results in the fully contracted state, is made possible by the lack of muscle, apart from that associated with and immediately under the tegument, and the presence of many vesicles.

This explanation also accounts for the observation that individuals with larger scolices and bothria tend to take on the form with the single opening more readily than smaller worms. The key relationship is the size and amount of radial muscle in the bothria as against the dimensions of the interbothrial mass, and the disposition of the latter is related partly to the state of contraction and thickness of the neck.

Nervous system

To Cooper's (1918) description and to our earlier description (Burt and Sandeman 1969) we would add the presence of a layer of longitudinal cords (diameter 2–4 μ) just exterior to the main dorsal and ventral longitudinal muscle fibers of the cortex of the strobila. The distance between the cords is about 30–50 μ. At intervals these cords are cross-connected horizontally and fine branches can be seen coming off at right angles towards the body surface. Small branches with only 2–6 fibers lead to neurons which are associated with the muscle cells in their immediate vicinity. These longitudinal nerve cords appear to continue into the scolex along with the longitudinal muscle layer with which they are associated, but the constriction and heavy concentration of muscle where the bothria and neck join made firm determination impossible. The main tracts and finer cords consist of a large number of extremely fine fibrils and have relatively few cells definitely associated with them. The cell bodies associated with the system are small, with a granular nucleoplasm but little cytoplasm, and lie to the outside of the cords. No lateral branches from the main longitudinal tracts were seen.

There is also an extensive system of giant fibers similar to those described by Pintner (1925, 1934). This is extensive and ramifies throughout the medulla of the neck (Fig. 5). It has tracts running forward into the scolex and continues backwards along the length of the worm in two main tracts on each side of the reproductive organs. Lateral branches go to the body organs in the region between adjacent sets of genitalia. Branches are also found throughout the parenchyma of the medulla and extending into the cortex. The main tracts appear to be fibrillar although the fibrils are ill-defined; in many preparations the interiors of the tracts appear granular and may be neurosecretory.

Much of the description of Schneider (1902), especially the reference to the "Anschwellung," is more applicable to the giant system we have described than the conventional nervous system described by Cooper (1918).

Osmoregulatory system

The shape, size, and arrangement of the excretory vessels is variable. We agree with previous authors e.g. Cooper (1918), that in the mature regions of the worm there are four main longitudinal vessels. On each side there is a vessel at the extreme margin of the medulla, the other two are more internal and run just outside of the main body organs. In most of the specimens from salmon the vessels are quite small, whereas in flounder and gammarid material the vessels are large and reach a diameter

of 40 μ. A large number of anastomosing branches form a network which ramifies throughout the medulla and in the parenchyma between the body organs (Fig. 6). In addition, a third pair of smaller vessels runs between and parallel to the two main vessels on each side but lies nearer the more internal of the two. Towards the anterior end of the strobila, this third pair of vessels enlarges and may surpass, in size, the other two pairs of vessels. At irregular intervals the outer of the main vessels on each side sends out branches to the tegument. Most of these branches appear to end blindly but others communicate with the exterior by a narrow pore.

In the neck region the two main vessels on each side unite by a number of lateral channels and continue forward as a network in the lateral margins of the interbothrial mass. Each of these networks anastomoses with its homolog of the other side, both by a network in the tip of the bothrial mass and by vessels which cross over at the level of the posterior ends of the suckers.

Each of the third pair of vessels divides in the neck region and the four vessels that result run forward into the outer, unattached walls of the suckers where they branch to form a double network (Fig. 7). This network ramifies throughout the unattached walls of the suckers and anastomoses with the other vessels in the lateral borders of the interbothrial mass. These networks are referred to as double because, in the unattached portions of the suckers, the system is in two interconnected layers; one nearer the external wall of the sucker, the other near the internal wall. The central portion of the interbothrial mass has few vessels.

Also present throughout the whole scolex, and especially in the lateral borders of the anterior tip of the interbothrial mass, are large numbers of vesicles often containing calcareous corpuscles (Fig. 8). The quantity and size of the vesicles varies. Although they are present in the vicinity of excretory vessels no actual connections could be found.

Flame cells were best seen, by light microscopy, in silver-stained sections where the tapering bundle of cilia, 11–13 μ long, projected into a narrow duct 2–3 μ in diameter. Thin sections of the base of the flame cell examined with an electron microscope show that there are about 120 cilia. The fine vessels into which the cilia project have a highly convoluted inner wall.

Tegument

Surrounding the tegument of all the worms from gammarids was a mucouslike sheath which varied in thickness from 1.1 to 1.4 μ. It is most clearly evident in electron micrographs (Fig. 9 and 11). Since there is no limiting membrane around the outside, the sheath probably represents a protective layer secreted by the worm while in situ in its host. This sheath was lost following experimental infestation in fish hosts and was not seen in any worm examined from other fish hosts.

The tegument exists at two levels: a syncitial surface layer of cytoplasm without nuclei, which is joined by tubules to an inner layer comprising nucleated areas of cytoplasm between the parenchyma cells. The outer layer is thrown out into microtriches about 4μ long (Fig. 9) each having a proximal tubular portion (ca 0.1 μ diameter) and an electron dense distal portion (Fig. 10). Particularly evident in the distal portion is what appears to be a double membrane, the space between the membranes being fairly constant at 110–125 Å. The proximal part is clearly continuous with the outer cytoplasmic layer of the tegument and the membrane wall of the microtriches can be seen as a continuation of the outer membrane bounding the tegument. The outer cytoplasmic layer of the tegument appears to be densely granular but also contains several vesicles, and is particularly rich in mitochondria in its more proximal portion. Vesicles of various sizes can be seen in the outer portion of this layer and also between the microtriches. The tegument of the scolex shows the same organization as that of the strobila but there were more, and larger, vesicles in the scolex (Fig. 11). The organization of the tegument with its tegumental cells can be seen in Fig. 12; the outer sheath has been lost and the microtriches are freely exposed.

Underneath the outer tegumental layer is a basal lamella which is traversed by canals linking the outer tegumental layer with the underlying tegumental cells. Separating the basal lamella from the thin sheath of circular muscle is a dense layer of minute fibrils plus some loosely scattered vesicles (Fig. 3). These fibrils were also scattered between the tegumental and parenchymal cells in other sections. The tegumental cells project deeply into the cortex and contain many mitochondria similar in size and shape to the mitochondria of the outer tegumental layer. The innermost portions of these tegumental cells frequently contain bodies surrounded by several electron dense concentric membranes. Golgi bodies were always present in close proximity.

Discussion of Ultrastructure

In reviewing the development of germinal layers of cestodes, Burt (1963) suggests that the outside layer of adult cestodes is derived from endoderm rather than ectoderm. This view was also held by

Schauinsland (1886) for the Bothriocephalidae, by Vergeer (1936) for *Diphyllobothrium latum*, and by Scott (1965) for *Paricterotaenia paradoxa*. Thus, the apparent similarity in structure between the tegument of cestodes and the intestinal epithelium of higher animals may be a reflection not only of a similarity of function but also of ontogenetic origin.

The nature of the sheath surrounding the tegument of those *Bothrimonus* recovered from the invertebrate gammarid hosts is not known. If worms were removed from the haemocoel of the gammarid and left for an hour or so in either sea water, fresh water, or "invertebrate saline" (2 parts sea water, 9 parts fresh water), this outer layer was sloughed off leaving the microtriches freely exposed as they are in worms recovered from the fish host. Monné (1960) concluded that an acid mucopolysaccharide was either present in the whole cuticle, or in the external cuticular lamella, or on the surface of the worms. Monné further points out that Esping (1957) has shown that mucopolysaccharides are able to inhibit various enzymes and thus reasons that in this way, both cestodes and trematodes in the gut of their definitive host are protected from the action of the various enzymes present in the intestine. In the same way, the intestinal epithelium itself is protected from the same enzymes by its own acid mucopolysaccharide layer. Although the enzymes present in the gammarid haemocoel may not resemble those in the gut of the fish host, it is not improbable that a mucopolysaccharide is nevertheless being produced by the cestode which, lying in a confined and closed system, eventually becomes surrounded by this material in the form of a sheath as described. The cestode grows rapidly while in the gammarid host (Sandeman and Burt 1972) and such a sheath may not only render the worm immune to destruction by the large enzyme molecules of the host, but may also be sufficiently permeable to allow passage of the nutriments required by the growing parasite.

The microtriches of both the worms from gammarids and from fish are long (ca. 4 μ) and do not undergo any visible change following experimental infestation of trout and plaice (Sandeman and Burt 1972) when compared with worms recovered from the haemocoel of gammarids. With the exception of the few (3%) elongated spatulate microtriches described by Berger and Mettrick (1971), the length of microtriches so far recorded for Cyclophyllidea (Rothman 1963; Beguin 1966; Morseth 1966; Baron 1968, 1971; Sakamoto and Sugimura 1970) is small in comparison with those from primitive cestodes (Threadgold 1965; Charles and Orr 1968; Morris and Finnegan 1969).

Numerous mitochondria in the proximal portion of the tegumental cytoplasm indicate a high degree of metabolic activity in the tegument, which is in keeping with the ability of this species to grow rapidly and mature while still in the invertebrate host. Mitochondria are not found in such concentrations in the tegument of those Cyclophyllidea with cysticercoid larvae (Baron 1971; Ubelaker et al. 1970; D. G. Crowe personal communication) whereas those still growing, and metabolically more active, such as the cysticercus larvae of *Taenia multiceps* and particularly the hydatid larva of *Echinococcus granulosus* (see Race et al. 1965 and Jha and Smyth 1969, respectively) do possess numerous mitochondria in the tegumental cytoplasm. Similarly in larval pseudophyllideans, where the plerocercoids continue to grow in the intermediate host and maintain a high degree of metabolic activity, it is not surprising to find large concentrations of mitochondria in the tegument of such worms as *Ligula intestinalis* and *Schistocephalus solidus* (Charles and Orr 1968) as well as in the present material.

Lamellated bodies are evident in the proximal region of the subtegumental cells. They are probably similar to the phospholipid bodies described by Threadgold (1965). The bodies described by Threadgold, however, do not appear to reach the size found in the present material. They bear a close resemblance to lamellated inclusion particles described by Dowling and Gibbons (1962) which were seen in the pigment epithelium of albino rats. They compare the lamellated bodies they found with so-called myeloid bodies described in pigment epithelium of various lower vertebrates by Porter and Yamada (1960) and Yamada (1961) but suggest that they are different. According to Karli (1954), pigment epithelial cells can ingest trypan blue particles and are phagocytic. Dowling and Gibbons (1962) suggest that the lamellated inclusion bodies may be responsible for the phagocytic function of the cell. Such an interpretation could also be applied in the present case where the tegumental cells, in which these lamellated bodies are found, must be responsible for the intake of nutriments by absorption through the outer tegument. Such absorption may occur by active transfer as shown by Lumsden (1965, 1966) or by phagocytosis or pinocytosis. The function of these lamellated bodies seen in our material, however, is not known.

The presence of vesicles between the microtriches suggests that the tegument has a secretory function although it is possible that some vesicles are being absorbed rather than secreted. Smyth (1963, 1964) working on in vitro cultivation of *Echinococcus granulosus* describes the secretion of small viscid droplets by the scolex and suggests that these droplets originated in a layer of granular cells lying just beneath the anterior tip of the rostellum. Smyth's

photograph scale indicates that the diameter of these viscid droplets is of the order of 6 μ. No mention is made by Smyth of any enclosing membranes around the droplets but the droplets secreted from the scolex of *E. granulosus* may be similar to those secreted by the scolex of *Bothrimonus*.

According to Wardle and McLeod (1952), calcareous corpuscles appear during the development of tapeworms at about the same time as the excretory or osmo-regulatory system of canals. Nieland and von Brand (1969) describe the formation of these corpuscles in *Taenia taeniaeformis* and compare their findings with those of other authors working on different animal groups (André and Fauré-Fremiet 1967, Protozoa; Erasmus 1967, and Martin and Bils 1964, Trematoda; and Gouranton 1968, Arthropoda). In *T. taeniaeformis*, Nieland and von Brand found only one corpuscle in any given cell but this does not apply to all cestodes as Fig. 8 shows several concretions in the process of being formed within what appears to be a single cell. In view of the size that these corpuscles have already attained and as their internal structure is quite similar to that of fully formed corpuscles, we do not think it likely that they could all coalesce into a single concretion. The various possible functions of these corpuscles are enumerated by Nieland and von Brand who also relate, tentatively, the dense rings of granular material to the mineral component of the corpuscle and homogeneous material to the mucopolysaccharides identified by Chowdhury et al. (1955) and von Brand et al. (1960). In our material, the corpuscles are more heavily concentrated in the scolex and neck region where there is also a greater concentration of flame cells and associated ducts. We have not been able to observe any direct association between the corpuscles and the duct system although a suggestion has been made by Howells (1969) that these ducts might function in the transport of materials, such as glucose, from one part of the worm to another. It is also possible that minerals and mucopolysaccharides may be transported by this system although in view of the presence of lateral pores, which connect the duct system to the exterior, it seems less likely that the flame cell system is an internal transport system than that it is either excretory or osmoregulatory.

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Influence of Trisodium Nitrilotriacetate on the Mortality, Growth, and Fecundity of the Freshwater Snail (*Helisoma trivolvis*) Through Four Generations

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FLANNAGAN, J. F. 1974. Influence of trisodium nitrilotriacetate on the mortality, growth, and fecundity of the freshwater snail (*Helisoma trivolvis*) through four generations. J. Fish. Res. Board Can. 31: 155–161.

Studies on four succeeding generations of the snail, *Helisoma trivolvis*, indicated that nominal concentrations of trisodium nitrilotriacetate up to 100 mg/liter were not acutely toxic. Concentrations above 25 mg/liter appeared to impose a stress on the growth and fecundity of the snails. However, the effects seemed to be lessened with each succeeding generation. At predicted environmental levels Na_3NTA is unlikely to have any lasting effect on euryplastic species of aquatic invertebrates, such as *H. trivolvis*.

FLANNAGAN, J. F. 1974. Influence of trisodium nitrilotriacetate on the mortality, growth, and fecundity of the freshwater snail (*Helisoma trivolvis*) through four generations. J. Fish. Res. Board Can. 31: 155–161.

Des études effectuées sur quatre générations de limaces, *Helisoma trivolvis*, démontrent que des concentrations nominales de nitrilotriacétate de sodium jusqu'à 100 mg/litre ne sont pas vivement toxiques. Des concentrations dépassant 25 mg/litre semblent imposer une contrainte à la croissance et à la fécondité des limaces, mais à un degré qui va en diminuant à chaque génération successive. Nous en concluons que Na_3NTA aux concentrations prédites pour l'environnement n'aura probablement pas d'effets sur les espèces euryplastiques d'invertébrés aquatiques tel *H. trivolvis*.

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THE TRISODIUM salt of nitrilotriacetic acid (Na_3NTA) is being widely substituted for phosphates as a builder in detergents. Since bacterial degradation of this chemical is inhibited at low temperatures (Rudd and Hamilton 1972), 2–20 mg NTA/liter could be discharged in sewage effluents (Flannagan 1971).

Estimates of the acute toxicity of NTA (Jancoric and Mann 1969) and of Na_3NTA (Flannagan 1971) to various macroinvertebrates indicated that observed mortality was largely due to pH changes. Buffered solutions of these compounds were not acutely toxic even at very high concentrations (>250 mg/liter) (Flannagan 1971).

The present study was designed to investigate the long-term effects of exposure to Na_3NTA solutions on the mortality, growth, and fecundity of the freshwater snail (*Helisoma trivolvis*).

Methods

Using a stock concentration of 50 g/liter and activated charcoal dechlorinated Winnipeg tap water (T.D.S. around 117 ppm), nominal concentrations of 0, 6.25, 12.5, 25, 50, and 100 mg/liter Na_3NTA were provided by a Mount and Brungs (1967) proportioned dilutor to six mixing chambers. The flows were then directed to experimental tanks each of which was divided into three compartments by glass partitions. The whole system was maintained at a constant temperature (23 C) with a 12-h light–12-h dark photoperiod. In each tank temperature was measured daily and dissolved oxygen, *p*H and Na_3NTA were analyzed weekly.

Sixty juvenile *H. trivolvis* (mean weight about 10 mg) were selected from stock cultures, divided into 6 groups of 10 with approximately the same mean weight. The groups were placed in the first compartment of the experimental tanks. The snails were fed $\frac{1}{10}$ of their body weight per day of Tetramin Staple fish food (Tetra-Werke, Melle, West Germany). Once a week the animals were removed from their tanks, allowed to dry for 5 min at room temperature (about 22 C), weighed individually, and returned to their tank. The resultant weights were used to calculate their food ration for the following week and were plotted as mean weight/concentration to measure growth rate.

Ten snails selected from the first offspring of each exposure group were weighed and transferred to the second compartment to form the F_1 generation, 10

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TABLE 1. Results of Na_3NTA, T°, D.O., and pH analyses, given as mean ± 1 SD.

	Stock	100 mg/liter	50 mg/liter	25 mg/liter	12.5 mg/liter	6.25 mg/liter	Control
Na_3NTA calculated by vol	50 g/liter	103.6	51.1	27.1	11.8	5.3	0
Na_3NTA analyses[a]	48.7±3.6	103±11.66	49.3±4.9	23.6±1.6	11.3±1.7	5.6±1.0	0
Dissolved oxygen		7.2±0.3	7.1±0.5	6.9±0.5	6.7±0.9	6.7±0.6	6.6±0.6
pH		7.4±0.2	7.5±0.2	7.4±0.2	7.4±0.3	7.5±0.2	7.5±0.3
Temperature C		23.1±0.9	23.6±0.9	23.6±0.9	23.7±0.9	23.4±0.9	23.2±0.9

[a]Murray and Povoledo (1971).

from the first offspring of the F_1 to form the F_2 generation, and 10 from the F_2 to form the F_3 generation. At the end of each month the remaining offspring were removed from the compartment and counted.

In a few cases mortality occurred during the egg-laying period and the fecundity results have been adjusted, using the formula given below, to permit direct comparison of these results:

$$\text{adjusted fecundity} = \text{number offspring produced} \times \frac{\text{standard number of egg-laying days}}{\text{actual number of egg-laying days}}$$

the standard number of egg-laying days being 600 and 1050 for P_1 (10 snails for 60 and 105 days, respectively) and 750 for F_1, F_2, and F_3 (10 snails for 75 days).

To ascertain whether any change in fecundity was due to reduced egg-laying or was associated with mortality of eggs or embryos, counts of egg masses were carried out for a 5-day period on the F_1 generation and compared to the total production of juveniles in that generation.

Results

The results of dissolved oxygen, temperature, pH, and Na_3NTA analyses are given in Table 1. Although some variations between the various tanks for any given parameter occurred, the deviations were, in all cases, small. Except for the Na_3NTA concentrations they cannot be correlated with the biological phenomena observed.

A typical growth curve for *H. trivolvis* (mean of 10 specimens from culture stock) is given in Fig. 1. The snails take approximately 25 days to reach 30 mg, at which the given growth curve starts. Since the growth curve is essentially sigmoid in shape snails in the 0–30-mg range could well be different ages (in days). Thus experimental groups of snails starting within this range could give rise to growth curves appearing to be different. To avoid this complication the comparative growth curves (Fig. 2) show only the straight line part of the growth curves, i.e. growth from 100 mg for 46 days (see Fig. 1). The growth results from the end of each experiment used to give the *t* values for comparison of mean size achieved in 120 days (Table 2, 3) do not suffer from this drawback, since by this age the snails' growth curves parallel the X-axis. Growth in the control situtations appears to progressively decrease from F_1 to F_2 and F_2 to F_3 (Fig. 2). However, Table 3 indicates that, by the end of the experiment, only the F_3 control is significantly smaller than the P_1 generation control ($P<0.05$). No significant growth differences were observed between the snails in 6.25 and 12.5 mg/liter concentration and their controls (Table 2) in any generation. In the

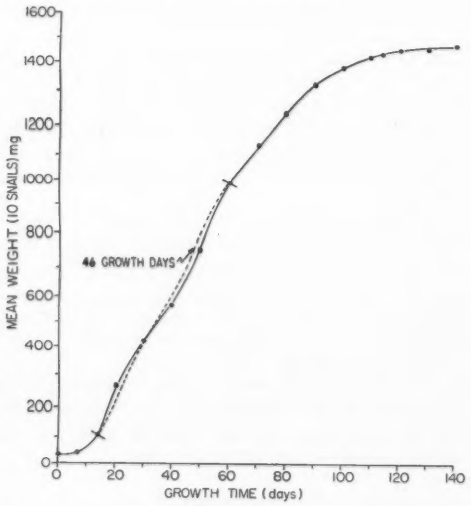

FIG. 1. Typical growth curve of laboratory population of *Helisoma trivolvis* (means for 10 specimens) indicating part of the curve used in comparison of growth rates.

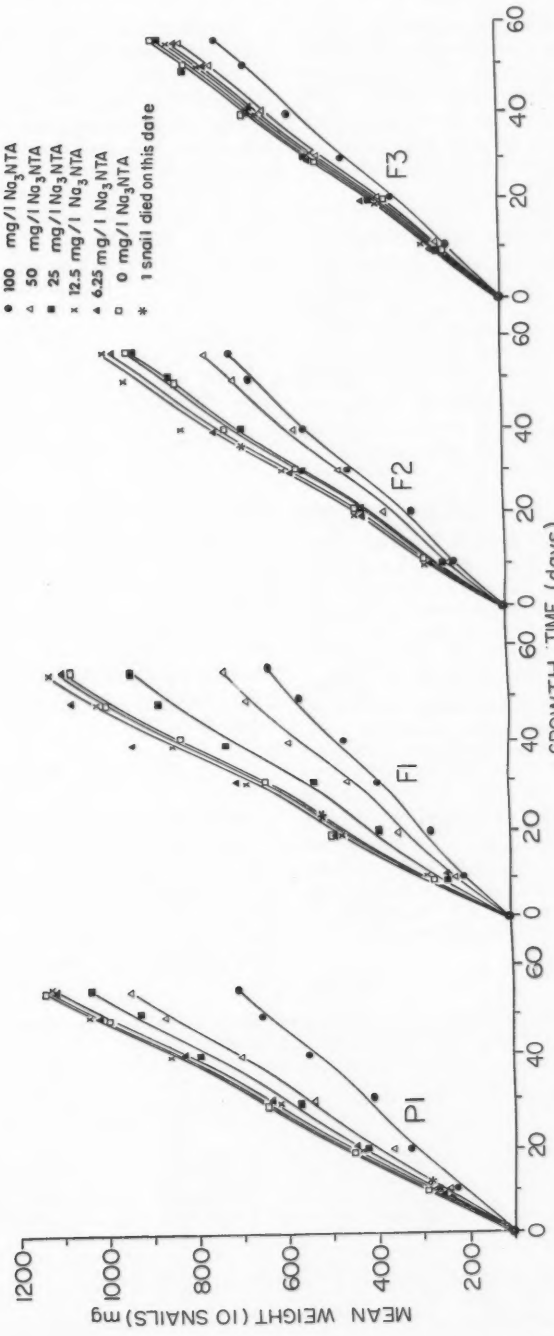

FIG. 2. Mean growth rates of four generations of *H. trivolvis* (10 per test) in various concentrations of Na_3NTA during the 46 days representeb by linear part of curve in Fig. 1.

25 mg/liter Na_3NTA only the F_1 snails were significantly smaller than their controls, while the animals in the 50 mg/liter concentration were significantly smaller than their controls in the P_1 ($P<0.05$) F_1 and F_2 ($P<0.01$), but not in the F_3 generation. In the 100 mg/liter tanks all four generations P_1, F_1, F_2 ($P<0.01$), and F_3 ($P<0.05$) were significantly smaller than their controls.

Comparisons within each generation of snails in the various experimental concentrations of Na_3NTA (Table 3, Fig. 2) show no significant differences in mean weight in either the 6.25 or 12.5 mg/liter Na_3NTA. However, in 25-, 50-, and 100-mg/liter concentrations the F_1 generation of snails is significantly smaller than the P_1 and no significant differences occur between the F_1 and F_2 generations. However, in the 50 and 100 mg/liter the F_3 generation is significantly larger than the F_2 ($P<0.05$) and in the 25-, 50-, and 100-mg/liter concentrations the F_3 has significantly higher mean weight than the F_1.

Figure 3 gives the number of offspring produced by each generation in the control and each concentration of Na_3NTA. These figures have been adjusted for mortality as outlined in the methods. Although statistical comparisons were not practicable, since they would involve keeping counts of eggs laid by each individual snail, the fecundity results appear to parallel the growth results in that in the control there seems to be a trend to produce fewer eggs with each succeeding generation, corresponding to the trend of decreased growth in Fig. 2. The snails in the 50- and 100-mg/liter concentrations of Na_3NTA show an initial drastic decrease in fecundity (to about 10% of control in the 100, and about 30% of control in the 50-mg/liter concentration) followed by a gradual recovery with each succeeding generation, corresponding to the trend, at these concentrations, for the growth to approach that of their control after an initial decrease.

Mortality among the experimental animals was quite rare, occurring generally among young adult snails and was attributable, in every case, to mishandling of the snails during the weighing procedure. Counts of egg masses produced in 5 days by the F_1 generation indicated that reductions in offspring produced by this generation as the concentrations of Na_3NTA increased were due to a reduction in the number of egg masses laid, not to mortality of juveniles. Numbers of offspring and egg masses, and the calculated numbers of offspring per egg mass were:

Concn (mg/liter)	control	6.25	12.5	25	50	100
Egg masses in 5 days	62	59	57	48	35	26
Total offspring by F_1 generation	3498	3000	2840	2480	1602	1352
Offspring per egg mass	56	51	50	52	46	52

Although not complete since the egg-mass count could be continued only for 5 days, these results indicate that, assuming a relatively constant egg-mass size, survival from egg to juvenile is consistent throughout the various concentrations (after that time the egg masses, particularly in the control tank, were too numerous to conveniently count, and removal of the mass from the tank walls would almost certainly result in death or serious damage to the embryo).

Discussion and Conclusions

Hübendick (1962) has shown that in freshwater habitats which are short-lived in evolutionary terms, the kind of specializations enabling many invertebrate species to be more successful than their competitors in particular biotopes are generally selected against inhololimnic animals. Gastropods have retained their adaptive plasticity and this

TABLE 2. t values for differences between mean size attained by the snails in the controls and various concentrations of Na_3NTA after 120 days of growth (see Table 3 for mean sizes).

	6.25 mg/Na_3NTA	12.5	25	50	100
P_1 control	0.1282 (17)[a]	0.0278 (17)	0.3802 (17)	2.4313[b] (17)	3.1824[c] (18)
F_1 control	0.8667 (17)	1.1035 (18)	1.8866[b] (18)	3.4615[c] (18)	3.8549[c]
F_2 control	0.2401 (19)	0.1428 (19)	1.3246 (18)	2.8926[c] (18)	4.3963[c]
F_3 control	0.5000 (19)	0.8397 (18)	1.0591 (19)	0.3186 (17)	1.8339[b]

[a]Degrees of freedom (number of snails in control + number in Na_3NTA–1).
[b]Difference significant at $P<0.05$.
[c]Difference significant at $P<0.01$.

TABLE 3. *t* values for differences between mean size attained between each generation by the snails in 120 days in the controls and the various concentrations of Na_3NTA. Mean size in grams attained by the snails in this period is listed before the *t* value.

	P_1v_1F	F_1vF_2	F_2vF_3	F_1vF_3	P_1vF_3
Control	(1.431, 1.275) 1.1470 (17)	(1.275, 1.343) 0.5218 (18)	(1.343, 1.228) 1.0777 (19)	(1.275, 1.228) 0.3903 (18)	(1.431, 1.228) 1.8238[b] (18)
6.25 mg/liter Na_3NTA	(1.413, 1.407) 0.0385 (17)[a]	(1.407, 1.365) 0.3658 (18)	(1.365, 1.288) 0.7216 (19)	(1.407, 1.288) 0.7970 (18)	-
12.5 mg/liter	(1.435, 1.447) 0.0974 (18)	(1.447, 1.361) 0.7603 (19)	(1.361, 1.304) 0.4943 (18)	(1.447, 1.304) 1.0983 (18)	-
25 mg/liter	(1.381, 1.145) 2.0866[b] (18)	(1.145, 1.201) 0.6113 (18)	(1.201, 1.314) 1.4468 (18)	(1.145, 1.314) 2.1638[a] (18)	-
50 mg/liter	(1.208, 0.870) 2.4653[b] (18)	(0.870, 0.982) 1.0099 (18)	(0.982, 1.265) 2.0702[b] (16)	(0.870, 1.265) 3.5330[a] (17)	-
100 mg/liter	(0.981, 0.839) 2.1962[b] (18)	(0.839, 0.906) 1.0601 (16)	(0.906, 1.075) 1.7807[b] (17)	(0.839, 1.075) 2.4106[a] (18)	-

[a]degrees of freedom.
[b]difference significant at $P<0.05$.
[c]difference significant at $P<0.01$.

according to Harman and Berg (1971), appears to be largely responsible for their successful colonization of such a wide variety of freshwater biotopes. *H. trivolvis* was collected from virtually every freshwater habitat sampled by Harman and Berg and is an excellent example of an euryplastic hololimnic species. The growth curves in Fig. 2 show a gradual decrease in growth in the control from generation to generation, partially substantiated by results in Table 3. A parallel reduction in fecundity can be seen in Fig. 3. Workers such as Chernin and Michelson (1957 a, b), Forbes and Crampton (1942 b), Hunter (1964), and others have shown, with various species of gastropods, that stresses such as crowding and extremes of environmental variables can produce effects similar to those shown. However, since the snails used in this study had ample space, were in running water, and were reared for all the "environmental" variables measured, in conditions very close to optimal (cf. Harman and Berg (1971), p. 29) it is unlikely they were reacting to a physical or chemical stress. The phenomena observed in the control if real, were possibly due to the intensive inbreeding brought about by the selection of only 10 experimental animals per generation, or could be the result of a nutritional deficiency.

Examination of the results on growth and fecundity of the snails in the three higher concentrations of Na_3NTA indicated it produced an immediate and decided stress. The maximum effect occurred in the first generation (F_1) to spend its whole life cycle in the Na_3NTA, followed by a gradual recovery in the succeeding two generations. Unfortunately the experiment was not carried on for one more generation since by the last generation (F_3) only the highest Na_3NTA concentration shows a marked effect which was apparent only in growth.

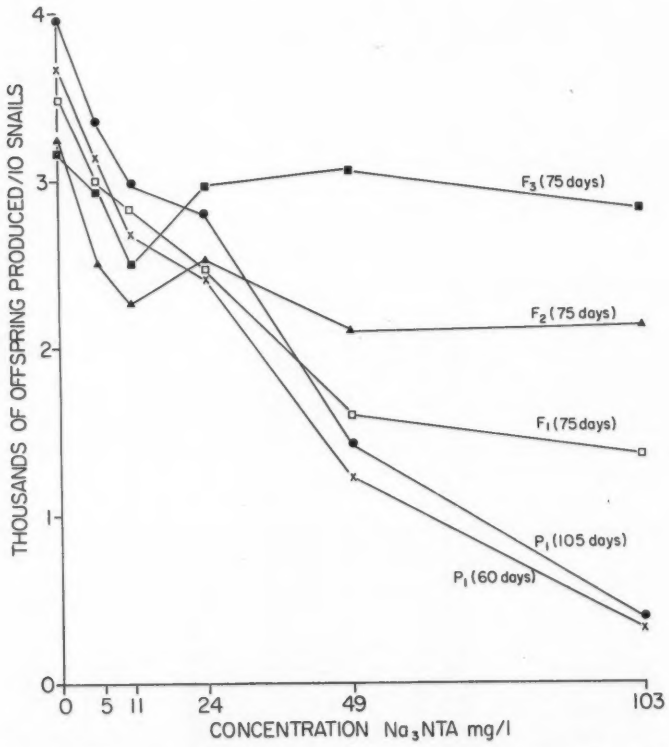

FIG. 3. Numbers of offspring produced by the four generations of *H. trivolvis* in various concentrations of Na_3NTA (adjusted for mortality). (Two figures are given for the P_1 to indicate the validity of using 75 days for the remaining three generations.)

In the intermediate concentrations of Na_3NTA, fecundity results particularly are more variable. This is presumably caused by individual snails within each population having slightly different threshold levels at which Na_3NTA stresses will be effected — a situation not unexpected in animals which have retained such a high degree of adaptive plasticity.

Possibly Na_3NTA directly affects only one of these functions (growth or fecundity) and indirectly the other. This could happen if large snails produced more or larger egg masses than small snails, or if snails which produced large numbers of eggs exhibited reduced growth. The evidence presented here suggests that the latter situation does not arise since smaller snails and reduced fecundity were both mainly associated with the higher concentrations of Na_3NTA. In the former case, although every egg mass was not closely examined, the egg masses appeared to be relatively small and to the naked eye appeared to contain a relatively constant number of eggs. There remains the possibility that larger snails produce larger numbers of egg masses and, although the recovery in growth and fecundity being not quite in phase. (F_3 100 mg/liter Na_3NTA snails significantly smaller than their control, while fecundity function apparently completely recovered), suggests there is not a direct relationship, this possibility must remain. Apparently *H. trivolvis* is able to adapt not only to natural environmental variables but also to the stress imposed on it by introduction of Na_3NTA into its environment. These experiments show that Na_3NTA is not lethal to *H. trivolvis* within the concentrations given. It does, however, appear to impose stresses especially at levels above 25 mg/liter, which *H. trivolvis* and presumably other euryplastic animals can accomodate in time. However, species of animals with less adaptive plasticity may not be able to recover from the stresses. Further experimentation, with stenoplastic species, seems essential if Na_3NTA is to be judged acceptable to the aquatic environment.

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Cleidodiscus baldwini n. sp. (Monogenoidea: Ancyrocephalinae) from the Gills of the Trout-Perch (*Percopsis omiscomaycus*) of Lake Huron[1]

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DECHTIAR, A. O. 1974. *Cleidodiscus baldwini* n. sp. (Monogenoidea: Ancyrocephalinae) from the gills of the trout-perch (*Percopsis omiscomaycus*) of Lake Huron. J. Fish. Res. Board Can. 31: 163–165.

A new species of monogenean, *Cleidodiscus baldwini*, from the trout-perch (*Percopsis omiscomaycus*) is described. This is the first report of a monogenean of the genus *Cleidodiscus* from North American populations of the trout-perch.

DECHTIAR, A. O. 1974. *Cleidodiscus baldwini* n. sp. (Monogenoidea: Ancyrocephalinae) from the gills of the trout-perch (*Percopsis omiscomaycus*) of Lake Huron. J. Fish. Res. Board Can. 31: 163–165.

L'auteur décrit une espèce nouvelle de monogénien, *Cleidodiscus baldwini*, trouvé chez l'omisco, *Percopsis omiscomaycus*. C'est la première mention d'un monogénien du genre *Cleidodiscus* chez des populations d'omiscos de l'Amérique du Nord.

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THIS paper is a continuation of our studies of parasites from fishes in Ontario waters. *Cleidodiscus baldwini* n. sp. were found on the gills of trout-perch from lakes Erie, Ontario, and Huron with higher incidence and intensity of infection in Lake Huron (type locality).

Methods

The hosts for the present study were collected from a variety of locations in the Great Lakes, but the parasite was encountered for the first time from trout-perch in South Bay (Lake Huron) during an investigation of this fish in 1969. The fish were captured in trawls and were held on ice until preliminary examination. The gills were removed and placed in a 1:4000 formalin solution according to the method of Putz and Hoffman (1963). After about 1 h the parasites were removed from the sediment for immediate study, or were preserved in a 5% formalin solution for later study. Twenty-five specimens of the new species, *C. baldwini*, and 10 specimens of the genus *Gyrodactylus* (from fins and body) were collected from 30 of 48 trout-perch. For description of the new species we used 10 specimens mounted in glycerin jelly according to the techniques of Mizelle and Klucka (1953). Measurements are given in millimeters; means are presented first, followed by minima and maxima in parentheses. Drawings were made with the aid of camera lucida, and in one case photomicrography was used.

Cleidodiscus baldwini n. sp.

Type host — *Percopsis omiscomaycus*, trout-perch.

Type locality — South Bay (Lake Huron).

Other localities — Lakes Ontario and Erie.

Location — Gills.

Specimens studied — Ten.

Type specimens — Holotype, U.S. Helm. Coll. No. 72802; two paratypes No. 72803; other paratypes in the author's collections.

Description — Small to moderate size species with smooth and thin cuticle. Body length is 0.547 (0.519–0.605) and greatest width is 0.173 (0.139–0.188). Eyespots approximately equal in size. Cephalic lobes well developed, two groups of head organs in each terminal lobe, and one group in each lateral lobe. Pharynx spherical, diameter

[1]Contribution No. 73-13 of the Fish and Wildlife Research Branch, Ontario Ministry of Natural Resources, Maple, Ont.

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0.053 (0.042–0.060), intestine confluent posteriorly. Peduncle usually short and broad; haptor slightly broader than long, greatest width 0.099 (0.060–0.109) and length 0.095 (0.058–0.107). There are two pairs of slightly unequal anchors, dorsal (Fig. 1) and ventral (Fig. 2). Each anchor base has a deep and superficial (slightly longer) root, and each has a shaft with anchor filament and point. Length of dorsal anchor is 0.041 (0.031–0.045), width of base is 0.017 (0.013–0.019). The dorsal bar is "V" shaped (Fig. 3). Its length is 0.030 (0.023–0.031) and width is 0.008 (0.006–0.009). Length of ventral anchor is 0.037 (0.031–0.042), basal part is 0.015 (0.013–0.016) wide. The ventral bar is straight with enlargement of both ends (Fig. 4), length is 0.027 (0.021–0.031) and width is 0.007 (0.006–0.008). The hooks (Fig. 5) are 14 in number, subequal, in normal arrangement (Mizelle 1938). Hook length 0.020 (0.016–0.021) pair 1, 17 (15–19); pair 2, 20–21; pair 3, 17 (15–20); pair 4, 20–21; pair 5, 16 (15–19); pair 6, 17 (15–19); pair 7, 19 (16–21). The copulatory complex consists of a cirrus and accessory piece articulated with the base of the cirrus. The cirrus (Fig. 6, 7) is a straight, sclerotized tube slightly attenuated distally, expanding terminally, and without a cirral thread, 0.032 (0.031–0.035) long. Accessory piece (Fig. 8, 9) solid, divided into two short rami distally, forming a fork through which the cirrus passes, 0.023 (0.022–0.028) long. The egg is oval and measures 0.083 (0.058–0.170) in length, and 0.056 (0.040–0.072) in width (Fig. 10).

Cleidodiscus baldwini n. sp. (Fig. 11) is named in memory of my colleague Norman S. Baldwin, scientist — research supervisor, Fisheries Section, Maple (1948–1957), Ont.

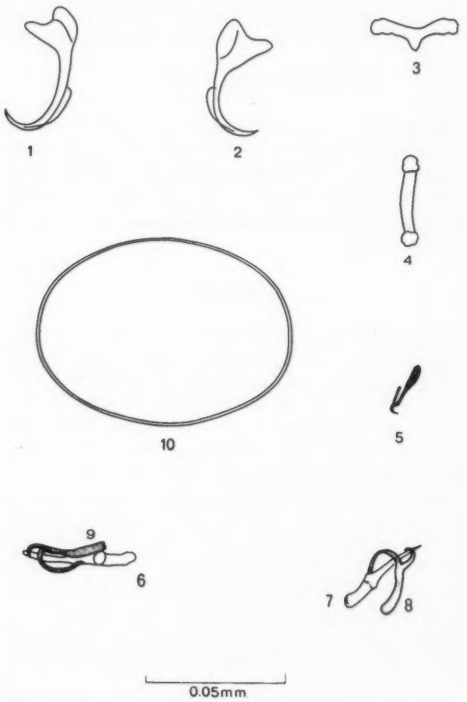
The nearest relatives of *C. baldwini* n. sp. are: *C. aculeatus* (Van Cleave and Mueller 1932) new comb. Mizelle and Regensberger 1945. Syn. *Ancyrocephalus aculeatus* (Van Cleave and Mueller 1932) Mueller 1934; *Urocleidus adspectus* Mueller, 1936. Syn. *Cleidodiscus* sp. Mizelle and Donahue 1944; *Urocleidus chrysops* Mizelle and Klucka 1953, and *U. adsimulatus* described by Mayes (1973). The copulatory complex in the last species closely resembled that of *C. baldwini* n. sp., though the haptoral armament was very different.

Remarks

The new species can be readily distinguished from *C. aculeatus* and *U. adspectus* by a radical difference in the haptoral armament. *C. baldwini* n. sp. differs from *U. chrysops* as follows: (1) the cirrus in the new species is smaller and does not have a cirral thread; (2) the accessory piece is smaller, different in shape, and articulated with cirrus; (3) the hooks are subequal in size and uniform in shape, with the accessory sclerotized process while in *U. chrysops* the hooks are not uniform in shape and size, and the sclerotized processes are absent; (4) the anchors in the new species are similar in shape but markedly different in size (smaller) especially ventral (about twice as small as in *U. chrysops*).

The new species differs from *U. adsimulatus* as follows: (1) the cirrus in the new species is twice as large; (2) the accessory piece is more than twice as large, and articulated to the base of the cirrus; (3) the anchors and hooks are larger and markedly different in shape; (4) the bars are markedly different in size.

On the basis of a study of the literature, and comparison of the presently reported new species with previously described species of the genera *Cleidodiscus* and *Urocleidus*, I find these genera are nearly identical and this tends to strengthen the



FIGURES 1–10. *Cleidodiscus baldmini* n.sp.
FIG. 1. Dorsal anchor; FIG. 2. Ventral anchor; FIG. 3. Dorsal bar; FIG. 4. Ventral bar; FIG. 5. Hook; FIG. 6 and 7. Cirrus; FIG. 8 and 9. Accessory piece; FIG. 10. Egg.

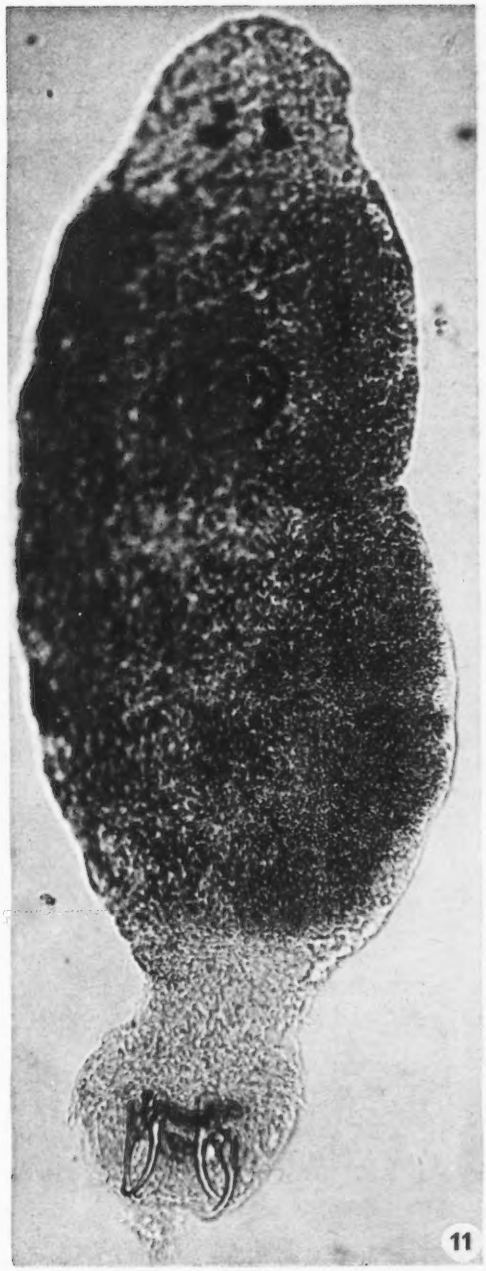


FIG. 11. *Cleidodiscus baldwini* n. sp. dorsal view of monogenean (×40).

position taken by Price (1957) and Price and Mura (1969). I believe, with Bychowsky and Nagibina (1970), that further study of the comparative morphology of species of the genus *Cleidodiscus* will result in transfer of many to the genus *Ancyrocephalus* (Creplin 1839) because these monogeneans are parasites of fish of the family Centrarchidae which is closely related to the family Percidae.

Acknowledgments

The author is grateful to Mrs E. Jermolajev, who made the original drawing, to Miss J. F. Robinson for taking the photographs, and to Mr J. A. Reckahn, for his kindness in supplying fish hosts. Thanks are due to A. H. Lawrie, Research Supervisor, for his help with the manuscript.

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Productivity and Production:Biomass Ratios of Bivalve and Gastropod Populations in an Eastern Canadian Estuary

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BURKE, M. V., AND K. H. MANN. 1974. Productivity and production:biomass ratios of bivalve and gastropod populations in an eastern Canadian estuary. J. Fish. Res. Board Can. 31: 167–177.

In a narrow, shallow estuary on the east coast of Canada, the dominant intertidal invertebrates were bivalve and gastropod molluscs. On a sand flat *Mya arenaria* produced 11.6 $g \cdot m^{-2} \cdot yr^{-1}$ flesh dry weight with a production:biomass ratio of 2.54, whereas *Macoma balthica* produced 1.93$g \cdot m^{-2} \cdot yr^{-1}$ with a P:B ratio of 1.53. On a *Spartina* marsh, *Littorina saxatilis* produced 3.25 $g \cdot m^{-2} \cdot yr^{-1}$ with a P:B ratio of 4.11. Approximate P:B ratios were applied to biomass figures for four other species to give the following estimates of productivity: *Mytilus edulis* on *Zostera* beds 19.7 $g \cdot m^{-2} \cdot yr^{-1}$ flesh dry weight; *M. edulis* on *Spartina* beds 3.5 $g \cdot m^{-2} \cdot yr^{-1}$; *Nassarius obsoletus* 1.15 $g \cdot m^{-2} \cdot yr^{-1}$; *Melampus lineatus* 1.1 $g \cdot m^{-2} \cdot yr^{-1}$; *Lacuna vincta* 0.06 $g \cdot m^{-2} \cdot yr^{-1}$. A total production of the molluscs in the estuary is estimated at 4.7% of the production of *Spartina* and *Zostera* (all measured in kcal). It is postulated that molluscs are the chief primary consumers in the inlet.

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Les invertébrés dominants de la région intercotidale d'un estuaire étroit et peu profond de la côte est du Canada sont des mollusques bivalves et gastéropodes. Sur un banc de sable, *Mya arenaria* produit 11.6 $g \cdot m^{-2} \cdot an^{-1}$ poids sec de chair avec un rapport production:biomasse de 2.54, tandis que *Macoma balthica* produit 1.93 $g \cdot m^{-2} \cdot an^{-1}$ avec un rapport P:B de 1.53. *Littorina saxatilis* dans un marais de *Spartina* produit 3.25 $g \cdot m^{-1} \cdot an^{-1}$ avec un rapport P:B de 4.11. On appliqué les rapports approximatifs P:B aux données de biomasse pour quatre autres espèces afin d'obtenir les évaluations de productivité suivantes: *Mytilus edulis* dans la zone de *Zostera* 19.7 $g \cdot m \cdot^{-2} \cdot an^{-1}$ poids sec de chair; *edulis* dans la zone de *Spartina* 3.5 $g \cdot m^{-2} \cdot an^{-1}$; *Nassarius obsoletus* 1.15 $g \cdot m^{-2} \cdot an^{-1}$; *Melampus lineatus* 1.1 $g \cdot m \cdot^{-2} an^{-1}$; *Lacuna vincta* 0.06 $g \cdot m^{-2} \cdot an^{-2}$. On évalue la production totale de mollusques dans l'estuaire à 4.7% de la production de *Spartina* et de *Zostera* (toutes mesures en kcal). On considère comme possible que les mollusques soient les herbivores principaux dans l'estuaire.

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AN ESTIMATE of productivity for each element in a food chain is essential to the formulation of any dynamic concept of an ecosystem. The measurement of productivity over long periods of time is tedious and difficult, whereas biomass measurements are simpler. If the rules governing the relation between production and biomass can be formulated, it should be possible to calculate production from biomass. The annual P:B ratio has been determined for only a few benthic marine organisms, (Peer 1970; Richards and Riley 1967; Sanders 1956; Hughes 1970) and this paper records P:B ratios for three additional species. It also gives estimates of the total productivity of the dominant herbivores, the molluscs, in the intertidal area of an estuary, and compares them with productivity of the marsh grass and eel grass beds in which they live.

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Methods

Petpeswick Inlet is on the eastern coast of Nova Scotia (44°45′N, 63°10′W) (Fig. 1). It is narrow and

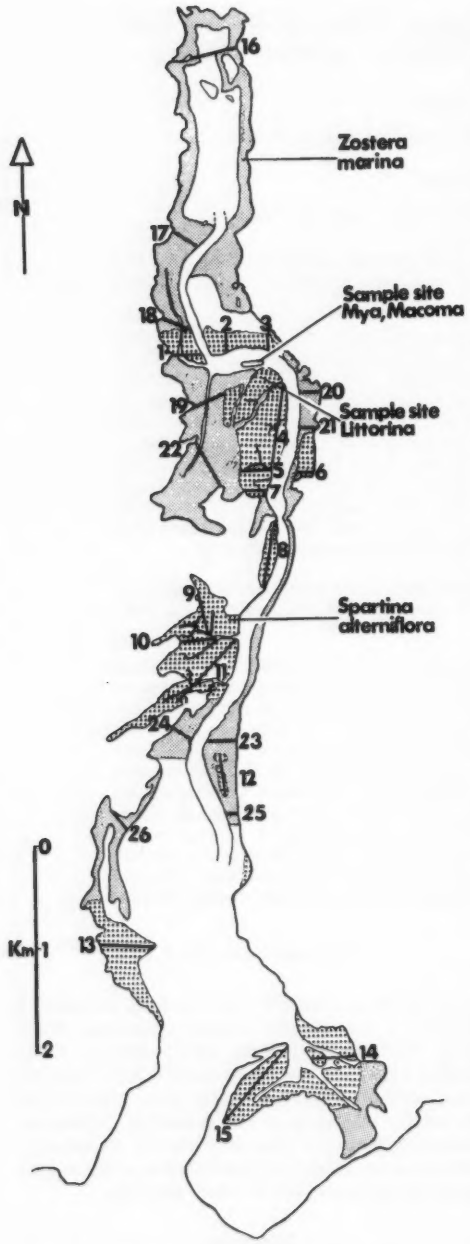

FIG. 1. Principal areas of vegetation, location of transects, and sampling sites in Petpeswick Inlet.

shallow with a bottom mainly of soft sediments and silt. Tides are of low amplitude (about 2 m) and at low tide about 60% of the area is exposed, revealing mud flats, sand flats, and extensive areas of macrophytes. About 0.8 km^2 is occupied by *Spartina alterniflora* and 1.85 km^2 by *Zostera marina*. There is also a considerable area of *Zostera* subtidally.

The bivalves *Mya arenaria* and *Macoma balthica* were sampled from a 48 × 40 m portion of a sand flat (Fig. 1). A grid of 16 rectangles, each measuring 12 × 10 m, was staked out and by pacing random coordinates, four monthly quadrat samples of 1/20 m^2 were taken in each, from April to December 1972. Each sample was dug to a depth of 15 cm; the top 2 cm were sorted through a Canadian Standard Sieve Series #25 (0.707-mm mesh) to obtain newly settled clams, and the remainder through a 46 × 34 cm nylon sieve of mesh size 1 mm.

The gastropod *Littorina saxatilis* was sampled from an irregularly shaped *Spartina* bed (Fig. 1). The habitat was divided into three zones and a total of 64 monthly 1/20 m^2 samples were taken by self-weighted stratified random sampling (Southwood 1966). Eighteen samples were retained for a size-frequency analysis, the remainder were counted and discarded.

The macrophyte beds were sampled in August 1971 to obtain biomass estimates of the key mollusc species. Twenty-six transects were laid out as in Fig. 1 and one sample of 1/20 m^2 was taken every 30 m along the transect. Transects 16–19 and 22 in *Zostera* were subtidal and were sampled by SCUBA divers.

Length of shells was determined with a sliding hand micrometer. In the bivalves the longest anteroposterior dimension was determined to the nearest 1 mm; in *Littorina* the distance from the base of the body whorl to the tip of the spire was measured to the nearest 0.5 mm. A monthly size-frequency distribution was determined for each species. In April, July, and October approximately 35 individuals of wide size-range were oven-dried at 65 C for 24 h and weighed to the nearest 0.01 mg. Regressions of total dry weight on length were calculated by the least-squares method. All samples from the August biomass survey were dried as above and weighed to the nearest 0.01 g.

The flesh was extracted from a subsample of each species and was weighed to obtain a flesh weight:total weight ratio in the subsample. This was then used to convert total dry weight of samples to flesh dry weight.

The cohorts in the populations of *Mya* and *Macoma* were isolated by measurements of annual rings on the valve, and by the probability paper method (Harding 1949; Cassie 1954). For *L. saxatilis*, only probability paper was used.

Subsamples of *Littorina* from May, August, September, and October 1972 were dissected to obtain estimates of the numbers of young snails in the brood pouches of the females.

Production was calculated by the methods set out in IBP Handbook No. 16 Chapter 12, (Crisp 1971). Elimination is the same as Crisp's "Mortality increment," but the use of the term elimination has become more widely adopted (e.g. Winberg 1971).

Results

Growth and production of *Mya arenaria* and *Macoma balthica*

In both *Mya* and *Macoma* the spring population consisted of two main year-classes and remnants of a third; in August newly settled clams appeared (Fig. 2, 3). Recruitment in the *Mya* population continued until November, at which time recruits comprised 85% of the sample. *Macoma* recruits appeared only in August and comprised 15% of the sample. The 2+ year-class was numerically dominant at all times.

The regression of weight on length had differing slopes at different times of year (Table 1). On each occasion the correlation coefficients were 0.98 or better. These relations were used to convert length measurements to weight, and to plot the growth curves for each cohort (Fig. 4a and b). Growth in *Macoma* slowed markedly after July, whereas *Mya* continued to grow well until November. This difference was most marked in the new recruits.

Changes in population numbers of the three age-classes in *Mya* could be fitted together to represent a hypothetical life history (Fig. 4c) but those for *Macoma* could not (Fig. 4d). Mortality in *Mya* occurred mainly in late summer and fall, whereas mortality in *Macoma* was fairly constant throughout the year.

Monthly data on growth and mortality were integrated to give the production figures shown in Fig. 4e and f. Population productivity reached a maximum in July and biomass peaked a month later in both species. The annual pattern of biomass change is rather different in the two species. In *Macoma* growth production fell to a very low level in the fall, and population biomass was depleted by elimination. Growth continued throughout the fall in *Mya*, and production approximately balanced elimination, so that the population biomass remained at a high level to the end of the year.

The relationship of the various cohorts is shown in Fig. 5. To calculate the P:B ratios for the 1st yr of life in the clams, the production of the 0 year-class of 1972 (which appeared in August 1972) was added to the production of the 0 year-class of 1971 (present before August 1972) and divided by the mean biomass. Similar calculations were made for the 2nd yr of life (Table 2).

Mya arenaria produced 11.6 $g \cdot m^{-2} \cdot yr^{-1}$ flesh dry weight to give a population P:B ratio of 2.54. The P:B ratio was greatest during the 1st yr of life, slightly less during the 2nd, and probably less in

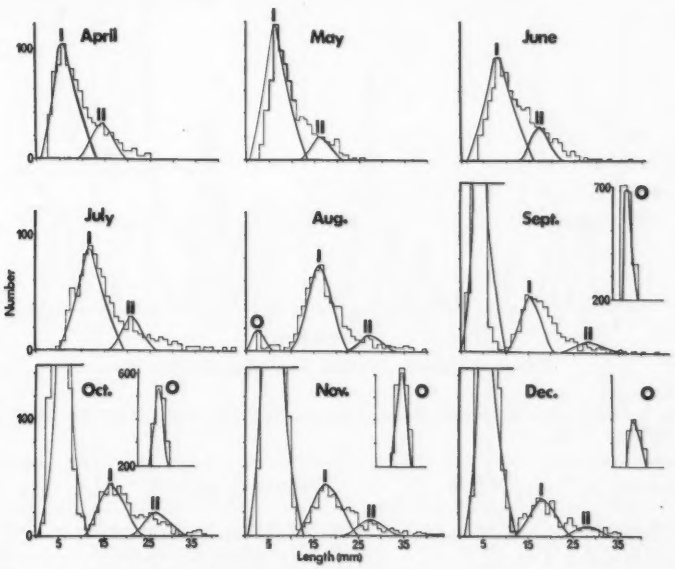

Fig. 2. *Mya arenaria*. Size-frequency distributions, April–December, 1972. Approximately normal curves for each year-class are indicated, and labelled 0, I, II. They were obtained by probability paper analysis and interpretation of annual rings.

the 3rd. The E:B ratio followed the same trend. *Macoma balthica* produced 1.93 $g \cdot m^{-2} \cdot yr^{-1}$ with a population P:B ratio of 1.53. *Macoma* also had the highest P:B ratio in the 1st yr, but the decline of this ratio in the 2nd yr was much more pronounced than in *Mya*, and yielded a population P:B that was much lower. Elimination exceeded production in *Macoma* but not in *Mya*.

Growth and production of *Littorina saxatilis*

There was no way of aging the shells of *Littorina*, since none appeared to live much over 1 yr. Hence, identification of age-classes (Fig. 6) is based only on the probability paper method (Harding 1949; Cassie 1954). In the overwintering population three cohorts appeared to be present. The largest speci-

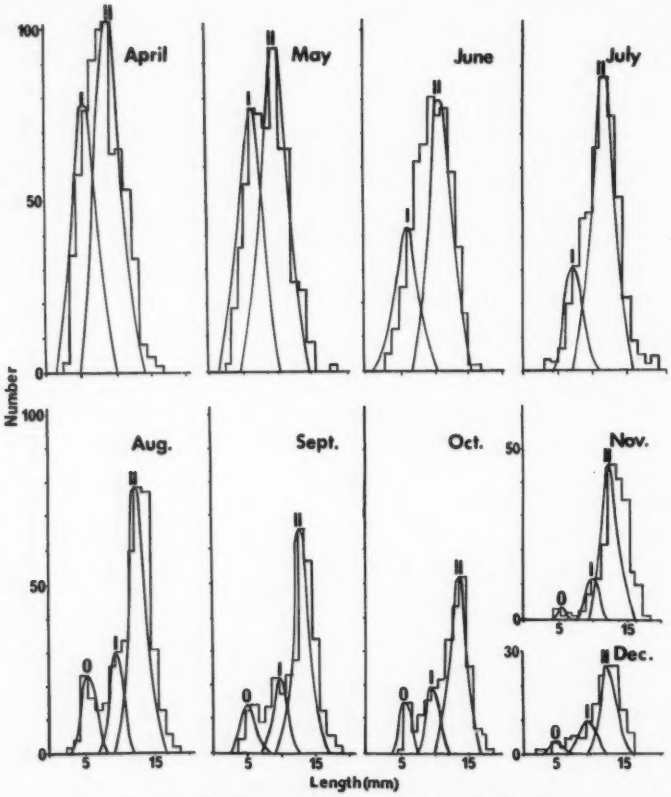

FIG. 3. *Macoma balthica*. Legend as for Fig. 2.

TABLE 1. Regression equations for total dry weight (W) on length (L) for April, July, and October 1972.

Species	Month	Regression equation	r
Mya arenaria	Apr.	$\log W = 2.589 \log L + \log 0.128$	0.991
	July	$\log W = 2.910 \log L + \log 0.039$	0.995
	Oct.	$\log W = 2.610 \log L + \log 0.115$	0.994
Macoma balthica	Apr.	$\log W = 2.917 \log L + \log 0.065$	0.983
	July	$\log W = 3.234 \log L + \log 0.032$	0.980
	Oct.	$\log W = 3.176 \log L + \log 0.037$	0.989

mens in May were incubating young, and new recruits appeared in the sample in June. Further recruitment was observed in August and October. Eggs, embryos, and young were being carried at all times between May and October by most snails longer than 4.5 mm. The best interpretation of

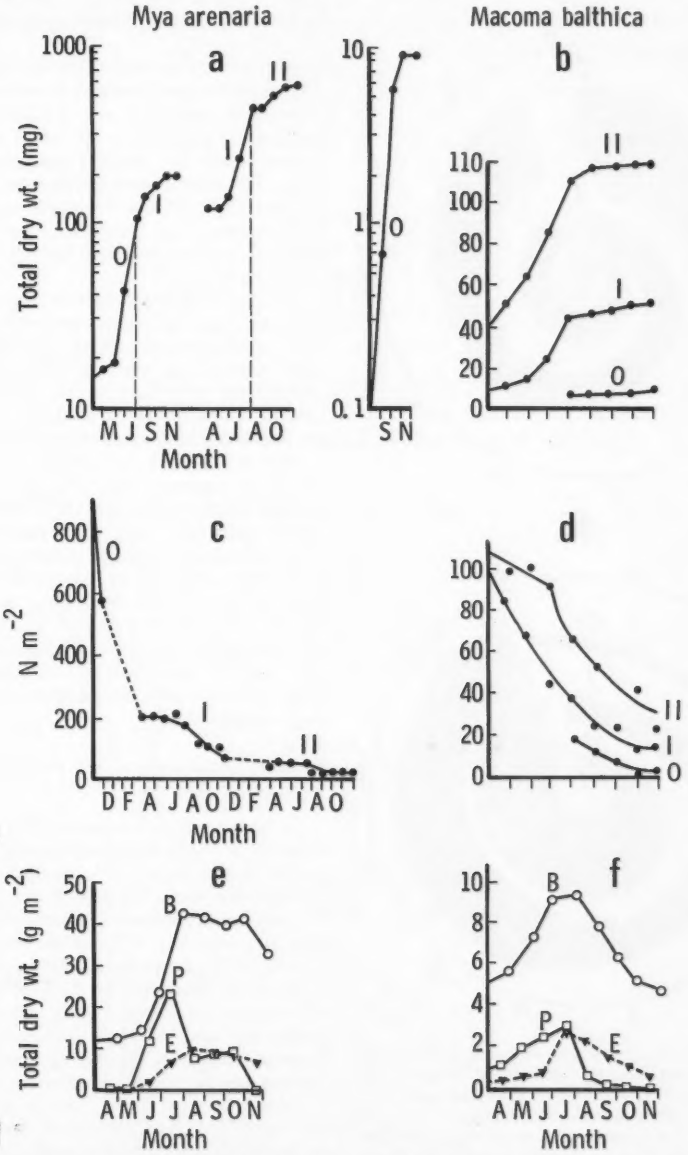

FIG. 4. *Mya arenaria* (left) and *Macoma balthica* (right). a, b, mean weights of year-classes; c, d, mean numbers in year-classes; e, f, biomass (B), production (P), and elimination (E) for the total population.

the data seems to be that the three overwintering cohorts were the recruits of June, August, and October and that each reproduced at the age of 1 yr. The equations for the regression of weight on length in April, July, and October were:

April $\log W = 2.668 \log L + \log 0.270$, $r = 0.988$
July $\log W = 3.163 \log L + \log 0.109$, $r = 0.984$
October $\log W = 2.778 \log L + \log 0.203$, $r = 0.984$

Growth curves for each cohort are shown in Fig. 7a. The apparent slow increase in mean weight of new recruits is probably an artifact resulting from prolonged recruitment of small specimens. The datum points for monthly density in each cohort (Fig. 7b) were smoothed by eye. Mortality was highest in the fall, but the very low density estimates in November and December may reflect the difficulty of finding specimens hidden in hollow stems or in the mud.

The first peak in production (Fig. 7c) is associated with the rapid growth of cohorts B and C, coupled with recruitment of cohort D, whereas the second peak is associated with the rapid growth of D, and recruitment of cohort E. The fall mortality mentioned above resulted in a high rate of elimination at this time, and a reduction in total biomass.

P:B ratios were calculated as previously for the June, August, and October broods (Fig. 8) and for the entire population (Table 3). The population produced 3.25 $g \cdot m^{-2} \cdot yr^{-1}$ to which the three cohorts contributed almost equally. As each cohort lives about 1 yr one would expect that the P:B ratios of each would be almost identical. Table 3 shows that although the ratios of cohorts one and three are virtually equal, the P:B ratio of cohort two was much less, because it had a fairly large biomass, but no production in winter and early spring.

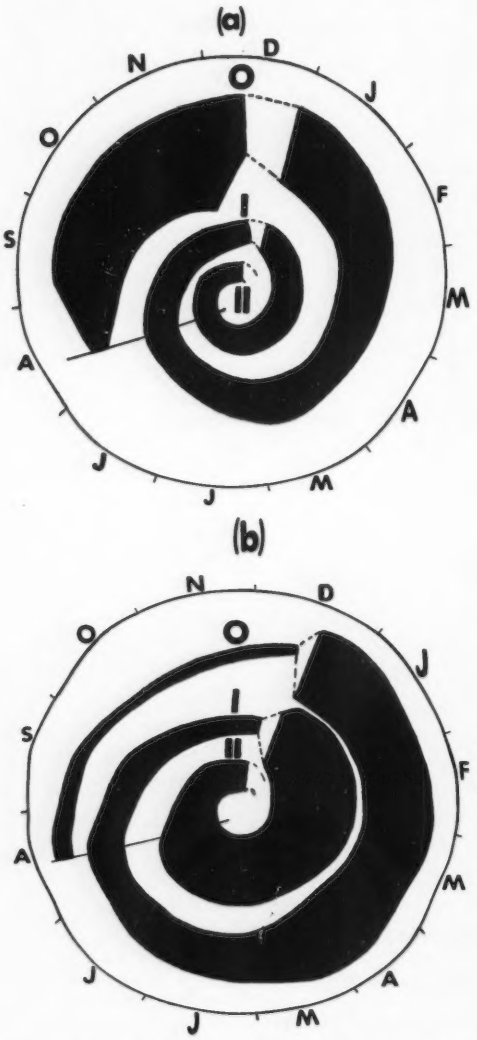

FIG. 5. *Mya arenaria* (a) and *Macoma balthica* (b). Life cycle diagrams showing relationship of age-classes based on data in Fig. 4 c and d. Width of spiral line approximately proportional to numbers present. Abrupt changes in thickness are a result of reconstructing a 3-yr life cycle from 1 yr's data, e.g. the 0+ group of *Macoma* born in 1972 were much less abundant than those born in 1971.

BIOMASS, DISTRIBUTION, AND PRODUCTION OF OTHER MOLLUSC SPECIES

In the quantitative survey of the distribution and biomass of the molluscs conducted in August 1971 in Petpeswick Inlet, *L. saxatilis* appeared in all *Spartina* transects and was absent from only a few of the 126 samples. It represented 57% of the numbers but only 19% of the dry flesh biomass (Table 4). By comparison, *Mytilus edulis* comprised 21% of the numbers and 63% of the biomass. In 65 samples on *Zostera* beds, *M. edulis* exhibited numerical and biomass dominance. *Melampus lineatus*, found in the higher levels of *Spartina*, *Lacuna vincta*, found on *Zostera*, and *Nassarius obsoletus*, sampled in the *Zostera*, were only locally abundant. No clear pattern of spatial distribution could be established for the molluscs as the biomass, abundance, and frequency of occurrence exhibited large fluctuations from transect to transect.

TABLE 2. Annual production(P), elimination(E), and average biomass(B) (as flesh dry weight) with ratios between them, for *Mya arenaria* and *Macoma balthica*.

Year-class	P $g \cdot m^{-2} \cdot yr^{-1}$	$\bar{B}$ $g \cdot m^{-2}$ ±SE	E $g \cdot m^{-2} \cdot yr^{-1}$	$P:\bar{B}$	$E:\bar{B}$
		Mya arenaria			
0	2.19	0.79±0.14	1.45	2.77	1.84
I	6.42	2.58±0.28	3.83	2.49	1.48
II (Measurable 5 mo only)	3.00	1.20±0.46	3.88		
Total	11.61	4.57±0.56[a]	9.16	2.54	2.01
		Macoma balthica			
0	0.23	0.11±0.03	0.16	2.09	1.45
I	1.15	0.69±0.13	0.51	1.67	0.73
II (Measurable 5 mo only)	0.55	0.46±0.17	1.39		
Total	1.93	1.26±0.22[a]	2.06	1.53	1.64

[a]Total SE = $\sqrt{\Sigma(SE_i)^2}$ where SE_i = standard error of the i^{th} year-class.

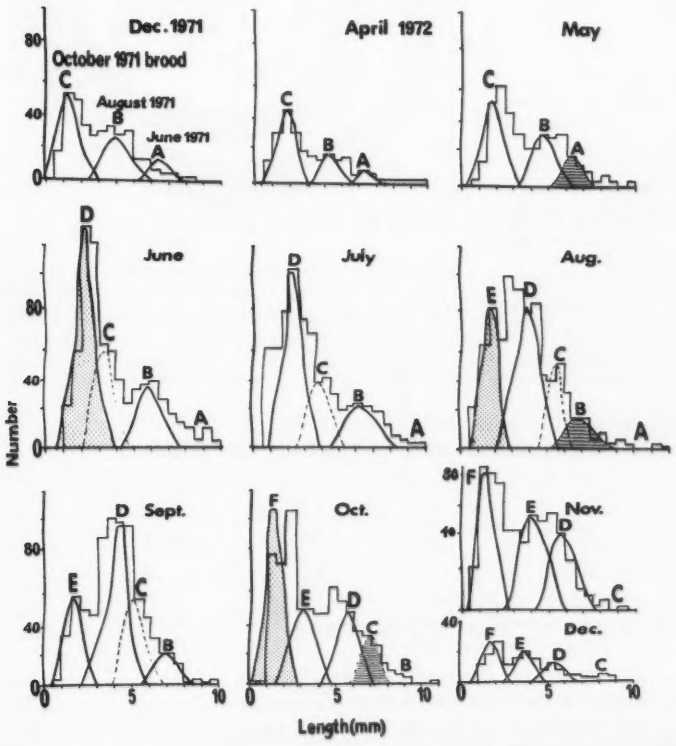

FIG. 6. *Littorina saxatilis*. Size-frequency distributions, December 1971 – December 1972. Approximately normal curves obtained by probability paper analysis for each cohort are indicated and labelled A–F.

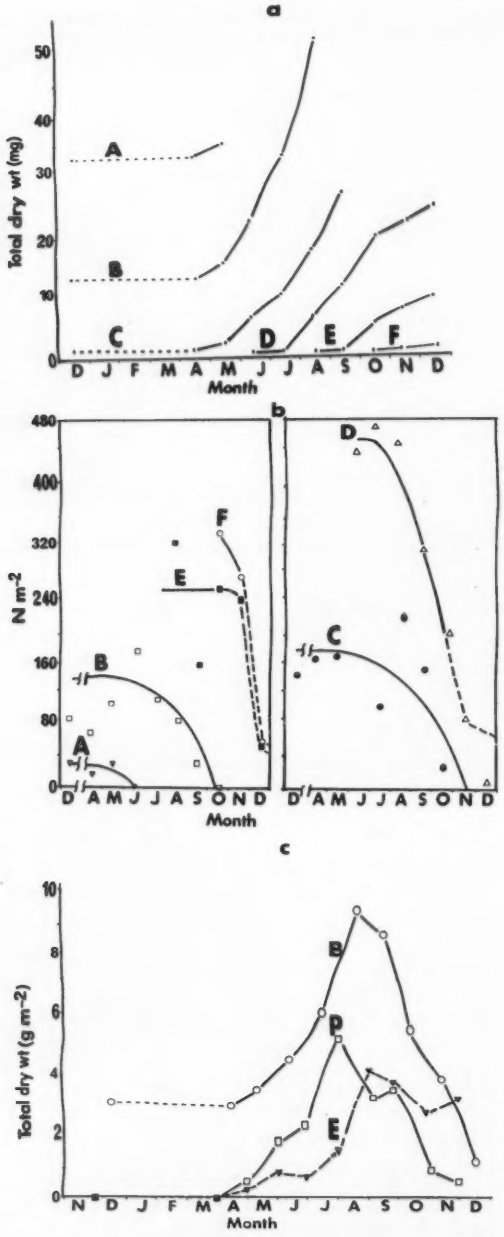

FIG. 7. *Littorina saxatilis*. a, mean weights of the cohorts; b, mean numbers in the cohorts; c, biomass (B), production (P), and elimination (E) for the total population, December 1971–December 1972.

The ratio of annual production to August biomass obtained for *Mya arenaria* and *Macoma balthica* (average = 1.29) and *Littorina saxatilis* (1.84) were applied as appropriate to the biomass figures obtained for the remaining species to obtain estimates of their annual production (Table 5). The production of *Lacuna*, an annual species, was calculated using the P:B ratio of 1.84, but that of the remaining species, those with a lifespan of a number of years, was calculated using 1.29. The productivity of molluscs on *Zostera* beds is about three times that on *Spartina*, because of the large biomass of *Mytilus*. The total production of molluscs on the macrophyte beds (Table 5) is 44 ± 19 metric tons flesh dry weight of which *Mytilus* comprises about 89%.

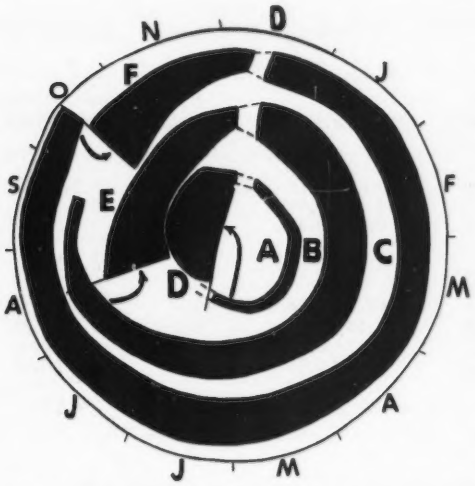

FIG. 8. *Littorina saxatilis*. Life cycle diagram showing relationship of cohorts, based on data in Fig. 7b. Width of spiral lines approximately proportional to numbers present. Abrupt changes in thickness are a result of reconstructing the life history from 1 yr's data. Arrows indicate breeding.

Discussion

The P:B ratios of *Macoma* and *Mya* agree fairly well with those obtained by Sanders (1956) for species living 2–3 yr. He found ratios of 1.99 and 2.28 for the molluscs *Pandora* and *Yoldia* and 0.94 and 2.16 for the annelids *Cistenoides* and *Nephthys*. On the other hand, Peer (1970) obtained a P:B ratio of 4.3 for *Pectinaria*, and Waters (1969) predicted on theoretical grounds that P:B values for species with this type of life history would be in the range 3.0–6.0. Soviet observations on barnacles, shrimps, and mussels, Hughes' (1970) study of bivalves living for 5 yr, and Birkett's (1959) study of *Mactra* all suggest annual P:B ratios less than 1.0 (Zaika 1972). There is a need for more data of the same kind before generalizations and predictions can be made. Care should be exercised in applying the *Mya* data to other populations, as those on the sand flats lived only 3 yr, and reached a maximum size of 35 mm, whereas many commercially exploited populations have a high proportion of larger, older specimens.

Littorina saxatilis lives for 1 yr, and the P:B ratio of 4.11 is similar to the values of 4.58 and 5.0 obtained for the annual forms *Ampharete* (Richards and Riley 1967) and *Ampelisca* (Sanders 1956). As predicted by Waters' generalizations about P:B ratios in the lifetime of a cohort, animals with shorter life histories have higher P:B ratios on an annual basis.

The total production of molluscs in the macrophyte beds, 1.76×10^8 kcal, may be compared with the estimated production of macrophyte material, 3.77×10^9 kcal (K. H. Mann and B. Hatcher unpublished data), indicating a transfer efficiency of 4.7%. However, such a comparison is incomplete,

TABLE 3. Annual production, elimination, and average biomass, (as flesh dry weight) with ratios between them, for *Littorina saxatilis*.

Cohort	P $g \cdot m^{-2} \cdot yr^{-1}$	$\bar{B}$ $g \cdot m^{-2}$ ±SE	E $g \cdot m^{-2} \cdot yr^{-1}$	P:$\bar{B}$	E:$\bar{B}$
1	0.81	0.16±0.06	0.27	5.08	1.69
2	1.00	0.34±0.07	0.47	2.94	1.37
3	1.44	0.29±0.07	1.34	4.92	4.60
Total	3.25	0.79±0.07[a]	2.08	4.11	2.31

[a]Total SE = $\sqrt{\Sigma(SE_i)^2}$ where SE_i = standard error of the i^{th} year-class.

as no account has been taken of production by planktonic and benthic algae or of production by molluscs living below mean low water.

Zaika (1972) uses the term specific production (= C) for an amount of production per unit of the system biomass. In an extensive review of the available information, C is tabulated on a daily basis for a wide range of organisms, and it is shown that the upper and lower limits of C are predictable from the length of life of the organism. However, in most cases the observations on rate of production are of short duration, so that extrapolation to annual rates is hardly possible.

Acknowledgments

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TABLE 4. Average density ($N \cdot m^{-2}$) and average flesh biomass ($g \cdot m^{-2}$) of each mollusc species on the macrophyte beds.

Habitat	Species	$N \cdot m^{-2}$	% of total	biomass $g \cdot m^{-2}$	% of total	Flesh biomass metric tons
Spartina	*L. saxatilis*	331±73	56.6	0.81±0.16	19.0	0.7±0.1
	M. edulis	125±47	21.4	2.69±1.81	63.0	2.2±1.5
	M. lineatus	129±63	22.0	0.77±0.39	18.0	0.6±0.3
	Total					3.5±1.5
Zostera	*M. edulis*	817±362	81.5	15.2±7.79	93.7	28±14
	L. saxatilis	140±82	14.0	0.10±0.04	0.6	0.2±0.07
	N. obsoletus	35±22	3.5	0.89±0.45	5.5	1.6±0.8
	L. vincta	10±6	1.0	0.03±0.01	0.2	0.06±0.02
	Total					30±14

TABLE 5. Production estimates (flesh dry weight) for each species on the macrophyte beds.

Species	Habitat	Production			
		$g \cdot m^{-2} \cdot yr^{-1}$	metric tons[a]	$kcal \cdot m^{-2} \cdot yr^{-1}$[b]	(*kcal*)
Mytilus edulis	*Spartina*	3.5±2.3	2.8±1.9	14±9.2	$1.12 \pm 0.76 \times 10^7$
Littorina saxatilis	*Spartina*	1.5±0.3	1.2±0.2	6.0±1.2	$4.80 \pm 0.80 \times 10^6$
Melampus lineatus	*Spartina*	1.1±0.6	0.9±0.5	4.4±2.4	$3.60 \pm 2.00 \times 10^6$
Total		6.5±2.4	4.9±2.0	24.4±9.6	$1.96 \pm 0.79 \times 10^7$
Mytilus edulis	*Zostera*	19.7±10.1	36±19	78±40	$1.44 \pm 0.76 \times 10^8$
Littorina saxatilis	*Zostera*	0.23±0.09	0.3±0.1	0.72±0.28	$1.20 \pm 0.40 \times 10^6$
Nassarius obsoletus	*Zostera*	1.15±0.56	2.1±1.1	4.60±2.24	$8.40 \pm 4.40 \times 10^6$
Lacuna vincta	*Zostera*	0.06±0.01	0.1±0.02	0.24±0.04	$4.00 \pm 0.80 \times 10^5$
		21.2±10.1	39±19	84±41	$1.54 \pm 0.76 \times 10^8$
Total			44±19		$1.76 \pm 0.76 \times 10^8$

[a]Production in whole nilet, on macrophyte beds.
[b]Based on a value of 4 kcal/g flesh dry weight obtained from the literature.

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Stress of Formalin Treatment in Juvenile Spring Chinook Salmon (*Oncorhynchus tshawytscha*) and Steelhead Trout (*Salmo gairdneri*)

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WEDEMEYER, G., AND W. T. YASUTAKE. 1974. Stress of formalin treatment in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 179–184.

The physiological stress of 200 ppm formalin treatments at 10 C is more severe in the juvenile steelhead trout (*Salmo gairdneri*) than in the spring chinook salmon (*Oncorhynchus tshawytscha*). In the steelhead, a marked hypochloremia follows a 1-hr treatment and recovery requires about 24 hr. During longer treatments, hypercholesterolemia together with reduced regulatory precision, hypercortisolemia, alkaline reserve depletion, and hypocapnia unaccompanied by a fall in blood *p*H occur — suggestive of compensated respiratory alkalosis. In the spring chinook, hypochloremia and reduced plasma cholesterol regulatory precision are the significant treatment side effects but recovery requires only a few hours.

Formalin treatments also cause epithelial separation, hypertrophy, and necrosis in the gills of both fishes but again, consistent with the physiological dysfunctions, these are more severe in the steelhead.

WEDEMEYER, G., AND W. T. YASUTAKE. 1974. Stress of formalin treatment in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 179–184.

Le stress physiologique de traitements à la formaline à 200 ppm à 10 C est plus sévère chez les jeunes truites steelhead (*Salmo gairdneri*) que chez le saumon chinook (*Oncorhynchus tshawytscha*) de printemps. Chez la truite steelhead, un traitement d'une heure produit une hypochlorémie marquée, et le rétablissement requiert environ 24 hr. Des traitements de plus longue durée causent une hypercholestérolémie, accompagnée d'une diminution de la précision régulatrice, d'une hypercortisolémie, d'un épuisement des réserves alcalines et d'une hypocapnie sans diminution du *p*H sanguin — ce qui suggère une alcalose respiratoire compensée. Chez le saumon chinook de printemps, l'hypochlorémie et l'affaiblissement de la précision régulatrice du cholestérol plasmatique sont les effets secondaires significatifs du traitement, mais le rétablissement ne requiert que quelques heures.

Le traitement à la formaline cause également des changements pathologiques dans les branchies des deux espèces (séparation de l'épithélium, hypertrophie et nécrose). Mais ici encore, tout comme pour les effets physiologiques, les changements pathologiques sont plus sérieux chez la truite steelhead.

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FORMALIN is widely used in fish husbandry to control such external parasites as *Costia* and *Gyrodactylus* spp. However, the usual 1-hr treatment at 167–250 ppm can be unexpectedly toxic and substantial mortalities may occur (Wood 1968). Experience has shown that rainbow trout (*Salmo gairdneri*) are among the most sensitive of the salmonid fishes in this regard, (Rucker et al. 1963; Wood 1968; Smith and Piper 1972). A comparison of the toxic side effects occurring when coho salmon (*Oncorhynchus kisutch*) and rainbow trout are given formalin treatments revealed that these are more severe in the trout and impose a considerable stress on homeostatic mechanisms (Wedemeyer 1971). Also, the gill pathological changes caused by formalin treatments are more severe in the trout than in the coho (Rucker 1962; Smith and Piper 1972).

More complete knowledge of the histological and physiological dysfunctions occurring in the other salmonids commonly given formalin treatments, and especially of the time needed for recovery, would aid in designing treatment regimens to minimize these toxic side effects, increase treatment

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efficacy, and be more suited to such newer fish-cultural techniques as water reuse systems for the control of rearing environments.

This study is a comparison of the extent and duration of the stress of formalin treatments in two additional hatchery-reared salmonids in which treatment toxicity can be a problem, the steelhead trout (*S. gairdneri*) and spring chinook salmon (*O. tshawytscha*).

Methods

Clinically normal juvenile spring chinook salmon and steelhead trout were treated with 200 ppm formalin, containing methanol as a preservative, for 1, 2, 4, or 6 hr in aerated water of 20 ppm total hardness ($CaCO_3$) at 10 C. Subsamples of 5–10 fish were taken at 0 and 1, 2, 4, 6, or 24 hr during or post treatments as appropriate. Gill arches were preserved in Bouin's solution and caudal artery blood samples taken for analysis. Plasma osmolality, HCO_3^-, Ca^{++}, Cl^-, total protein, hemoglobin (Hb), bilirubin, *p*H, and cholesterol measurements were used to evaluate acid-base balance, ionoregulation and gill, kidney, and liver function. Gill tissues were prepared and examined by standard histological methods. Activation of the pituitary–interrenal axis was evaluated by determining the extent of interrenal vitamin C depletion and by plasma cortisol determinations. All analyses except osmometry were done by previously described methods (Wedemeyer and Chatterton 1971). Plasma osmolality measurements were done at this laboratory and at the Sierra-Nevada Aquatic Research Laboratory through the courtesy of Dr G. Crowley.

Results and Discussion

Histopathology

The gill histological changes were qualitatively similar to those previously reported for formalin-treated coho salmon and rainbow trout (Rucker 1962; Smith and Piper 1972). The most noticeable pathological change was in the gill epithilium. Varying degrees of hypertrophy as well as epithelial separation (from the lamellar supporting cells) were observed in both the chinook salmon and steelhead trout. Much of the hypertrophy occurring was in the form of cloudy swelling and hydropic degeneration.

As expected, chinook salmon suffered considerably less tissue damage than the steelhead trout. The gills of the former, after a 1-hr treatment, showed moderate epithelial hypertrophy (Fig. 1a and b). Hypertrophic and degenerating epithelial cells appeared only after longer (4-hr) exposures. These cellular changes often gave an irregular, ragged appearance to the lamellar surface (Fig. 1c). Still longer exposures (6-hr) caused little additional gill damage but, the epithelial hypertrophy was more uniform and extensive (Fig. 1d) than in fish treated for a lesser time.

Formalin treatments caused consistently more gill damage in the steelhead than in the chinook. The gill pathological changes in steelhead treated for 1 hr were almost as extensive as those in the 6-hr chinook group (Fig. 2a). In the chinook, however, little or no epithelial separation was seen. Steelhead given longer formalin treatments (2, 4, 6 hr) showed progressively more gill damage. At 2 hr, degenerative changes as well as some necrosis were quite evident (Fig. 2b). After a 4- or 6-hr exposure, the hypertrophy that occurred in all groups was accompanied by more pyknotic and karyorrhetic epithelial nuclei. Another noticeable change was the epithelial separation (Fig. 2c and d).

Pathologic Physiology

Together with the gill histological changes, formalin treatments caused several metabolic disturbances in the steelhead and chinook.

In a previous study (Wedemeyer 1971), interrenal ascorbate depletion, an index of pituitary activation, was found in the rainbow trout during formalin treatments but not in the coho salmon. In this study, both the steelhead and chinook maintained relatively constant levels of interrenal vitamin C even during extended (6-hr) exposures implying that a lesser amount of pituitary activation occurs and thus, that treatment stress is less severe in these species than in the rainbow trout (Table 1). In support of this, hypercortisolemia occurred only in the steelhead and then only during prolonged treatments while in the chinook, no significant ($P = 0.05$) increase was seen (Fig. 3). Thus, the amount of neuroendocrine involvement during a 1-hr, 200-ppm formalin treatment is probably minimal in these two fishes.

Formalin treatments at 200 ppm also caused fluctuation in plasma chloride levels (Fig. 4). During an hour exposure, a statistically significant ($P = 0.05$) hypochloremia developed in both fishes suggesting that formalin treatments interfered somewhat with gill chloride cell function in these species as they do in the rainbow trout. However, a hypochloremia of this magnitude would probably have limited biological significance. Chloride normalization in the salmon was fairly rapid, but the steelhead apparently require up to 24 hr for recovery.

Since chloride regulation may also involve plasma bicarbonate levels due to Cl^- exchange for HCO_3^- across the gill epithelium, additional experiments to evaluate the alkaline reserve status during formalin treatments were performed (Table 2).

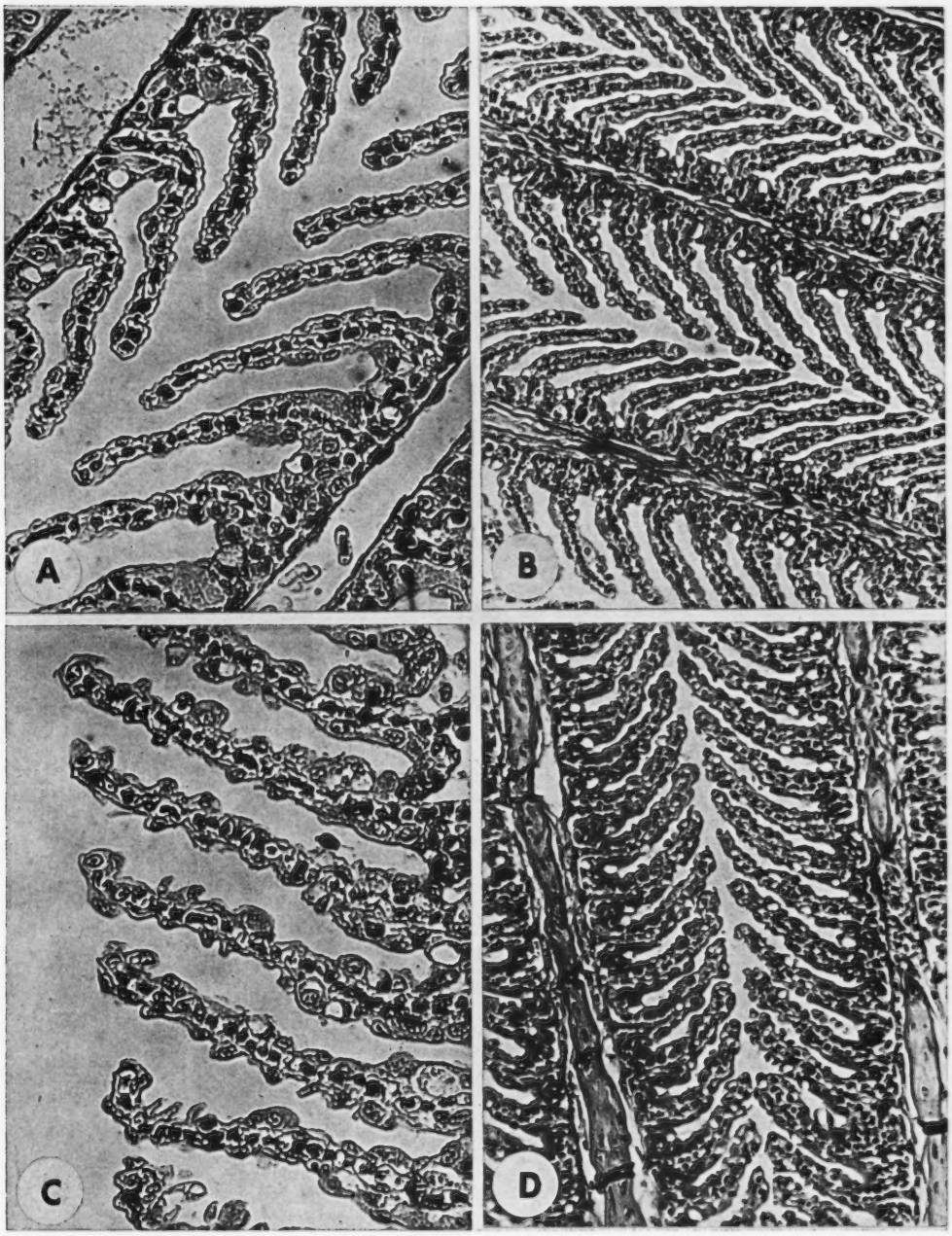

FIG. 1. Spring chinook salmon (*Oncorhynchus tshawytscha*) gill after exposure to 200-ppm formalin. H. & E. stained.
A, Untreated control, ×1300;
B, 1-hr exposure (note moderately hypertrophic gill epithelium), ×400;
C, 4-hr exposure (note hypertrophic and degenerating cells giving a ragged-appearing lamellar surface), ×2000;
D, 6-hr exposure (note epithelial cell with noticeably more hypertrophy, degeneration, and separation), ×400;

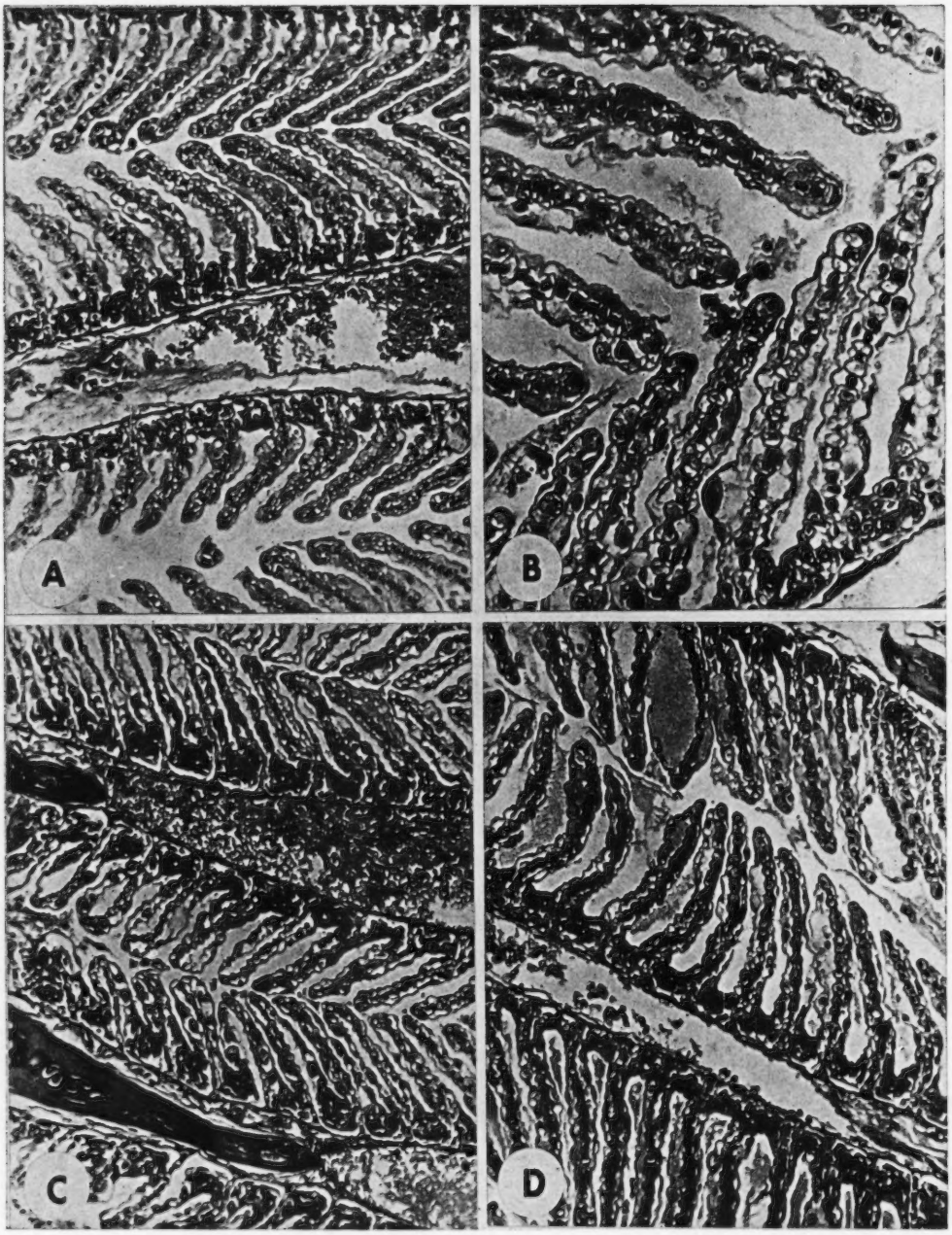

FIG. 2. Steelhead trout gill after exposure to 200-ppm formalin. H. & E. stained.
A, One-hour exposure (note hypertrophic lamellar epithelium), ×500;
B, 2-hr exposure (note cloudy swelling and hydropic degeneration of lamellar epithelium) ×950;
C, 4-hr exposure (note degenerative and necrotic lamellar epithelial separation from the lamellar supporting cells. ×400;
D, 6-hr exposure (note degenerative and necrotic lamellar epithelium and the epithelial separation accompanied by edematous fluid), ×450.

As shown by the blood *p*H, HCO_3^-, and derived P_{CO_2} values, only minor acid-base changes occurred even during a 2-hr exposure. Blood *p*H in the chinook fell slightly if the treatment time was extended but the decrease was not statistically significant, although in the steelhead even this slight decrease was not seen. However, consistent with the compromised ionoregulation in this species, the steelhead apparently maintained constant blood *p*H only at the expense of their alkaline reserve. Plasma HCO_3^- levels fell steadily if 200-ppm formalin treatments were extended past an hour.

Formalin exposure did affect other aspects of ionoregulation in these fishes, indicating that the gill pathological changes were functionally significant. Treatments at 200 ppm had no effect on plasma Ca^{++} regulation in the juvenile chinook, even when extended past 1 hr, but a significant ($P = 0.05$) hypercalcemia developed in the steelhead (Fig. 5). An elevated blood Ca^{++} level because of hyperproteinemia was ruled out by plasma protein determinations which showed that no significant total protein increase occurred. Mean ±SE plasma total protein levels (g/100 ml) during formalin treatments, 200 ppm, 10 C, 20 fish/sample, were:

Treatment time (*hr*)	Chinook salmon	Steelhead trout
0	4.0±0.2	6.4±0.2
1	4.4±0.3	6.6±0.1
2	4.0±0.2	5.5±0.3
4	4.3±0.1	6.9±0.3
6	4.5±0.2	5.5±0.2

Thus the hypercalcemia was not due to protein binding, indicating that calcium regulation in the steelhead was at least partially compromised.

To further evaluate the acid-base balance, the blood CO_2 status in these two fishes was compared by calculating approximate arterial P_{CO_2} levels from the previously obtained blood *p*H and HCO_3^- values using the Henderson-Hasselbalch equation (Table 2). Consistent with the hypochloremia and HCO_3^- depletion, (derived) arterial P_{CO_2} levels in the steelhead dropped significantly ($P = 0.05$) if the formalin treatments were extended past 1 hr. However, the blood *p*H remained higher than can be accounted for by this degree of hypocapnia suggesting that a component of respiratory alkalosis was superimposed on any metabolic acidosis present. Although it was not quantitated, a subjective judg-

TABLE 1. Interrenal vitamin C levels during 200-ppm formalin treatments, 10 C. Ascorbate value (mg/g) given as means ±SE, 10 fish/sample. The depletion that occurred was not statistically significant ($P = 0.05$).

Time (*hr*)	Chinook salmon	Steelhead trout
0	362±32	333±41
1	301±24	368±16
2	366±42	336±28
4	334±28	277±19
6	338±29	327±23

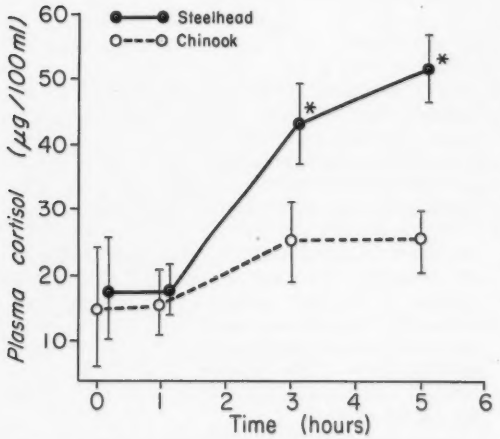

FIG. 3. Plasma cortisol ("stress hormone") levels during 200-ppm formalin treatments as a function of exposure time, 10 C. A 1-hr treatment had no effect. Data given as means ± SE, 20 fish/sample, *indicates significant difference from initial (control) values ($P = 0.05$).

TABLE 2. Blood *p*H, HCO_3^- and (derived) PCO_2 values of A) chinook salmon and B) steelhead trout during 200-ppm formalin treatments, 10 C, six fish/sample, values given as mean ±SE.

Time (*hr*)		*p*H	HCO_3^- (meq/l)	PCO_2 (mmHg)
0	A	7.83±.05	10.7±.7	6.6±.4
	B	7.63±.03	9.0±.3	8.8±.4
2	A	7.80±.06	9.7±.8	6.4±.1
	B	7.75±.07	8.1±.5	6.0±.5*
4	A	7.75±.04	9.8±.7	7.3±.6
	B	7.72±.08	7.4±.5*	5.9±.6*
6	A	7.72±.05	9.9±.7	8.0±.5
	B	7.68±.08	7.5±.5*	4.7±.4*

*Indicates significantly different from 0-hr levels.

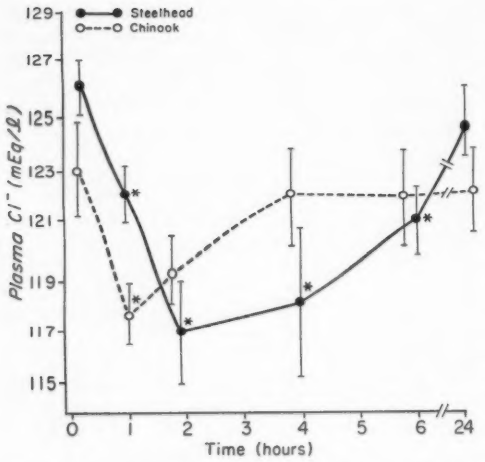

FIG. 4. Hypochloremia and recovery during and following 1-hr formalin treatments, 200 ppm, 10 C. Data given as mean X ± SE, 20 fish/sample. * indicates significant difference from initial (control) values ($P = 0.05$).

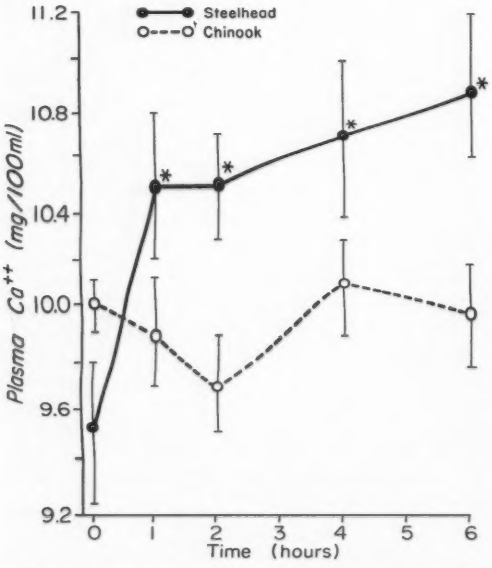

FIG. 5. Plasma calcium levels during 200-ppm formalin treatments as a function of exposure time, 10 C. Data given as mean X ± SE 20 fish/sample. * indicates significant difference from initial (control) values ($P = 0.05$).

ment was that gill ventillation rates did increase during formalin treatments. Consistent with this, Lanzing (1963) has reported that hyperventilation occurs when Whiting (*Sillago ciliata*) are given formalin treatments.

The occurrence of respiratory alkalosis would also be indirect support for the idea that the biotransformation of formaldehyde to formic acid, which was suggested in formalin treated rainbow trout (Wedemeyer 1971), may occur slowly or not at all in steelhead — a question which will have to await definitive blood formic acid analyses. For practical purposes, however, there probably would not be enough of the acid-base shift due to respiratory alkalosis to explain the immediate and delayed mortalities sometimes caused by formalin treatments unless an existing gill infection had compromised acid-base regulation. Perhaps the increased toxicity of formalin treatments to fish with myxobacterial gill disease or *Costia* sp. infections can be partly explained on this basis.

Somewhat surprisingly, the total plasma osmolality was not significantly affected by formalin treatments. Mean ±SE plasma osmolality (mOsm) during formalin treatments, 200 ppm, 10 C, was (number of fish in parentheses):

Treatment time (*hr*)	Chinook	Steelhead
0	313±5(8)	323±1(16)
1	300±5(10)	329±7(12)
2	312±7(9)	330±4(14)
4	321±4(13)	325±2(13)
6	311±6(11)	323±3(15)

Previous workers have found that exposure to toxicants (Cu^{++} and Zn^{++} which cause gill pathological changes) lowers plasma osmotic concentration while nonspecific stress can increase it (Lewis and Lewis 1971; Umminger 1970). In this case, the hypertrophy and separation of the epithelial layer from the lamellar supporting cells was evidently not severe enough to influence total plasma osmolality. However, the physiological and histological changes did correlate well in other respects. Consistent with the previous results with rainbow trout and coho salmon (Wedemeyer 1971), the steelhead showed both more extensive gill pathological changes and blood chemistry fluctuations than did the spring chinook.

To evaluate the effect of formalin treatments on aspects of steroid metabolism, plasma cholesterol levels were monitored. Formalin exposure for more than an hour caused a mild hypercholesterolemia in the steelhead but not in the chinook (Fig. 6). Significantly, the precision of blood cholesterol

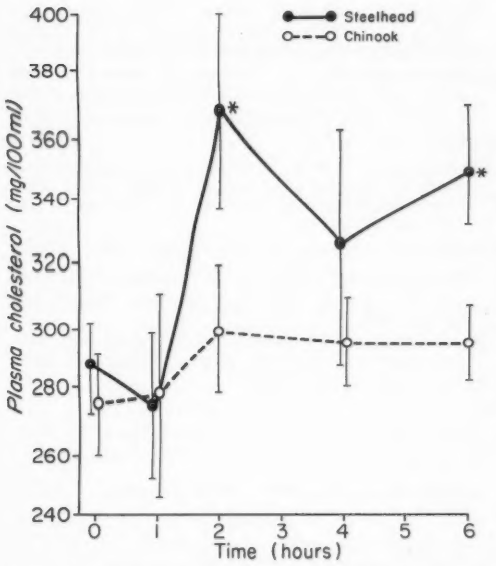

FIG. 6. Plasma chloesterol levels during 200-ppm formalin treatments as a function of exposure time, 10 C. Data given as mean X ± SE, 20 fish/sample. * indicates significant difference from initial (control) values (P – 0.05).

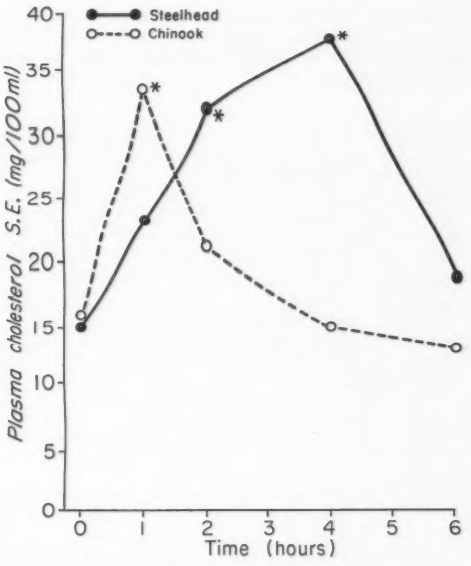

FIG. 7. Decreased plasma cholesterol regulatory precision during 200-ppm formalin treatments, 10 C. * indicates significant difference from initial (control) values (P = 0.05).

regulation was also affected by formalin, even in the chinook where no real change in average blood levels occurred (Fig. 7). Perhaps some of the well-known variability in the acute toxicity of formalin to fish (when death is used as the end point) is due to this kind of decreased homeostatic regulatory precision.

Total blood hemoglobin levels were not significantly affected by formalin treatments. Mean ±SE blood hemoglobin levels (g/100 ml) during formalin treatments, 200 ppm, 10 C, were (number of fish in parentheses):

Treatment time (*hr*)	Chinook salmon	Steelhead trout
0	9.5±0.2(8)	8.6±0.3(13)
1	9.3±0.3(8)	9.3±0.2(13)
2	10.1±0.3(8)	8.8±0.3(13)
4	9.9±0.3(8)	9.3±0.3(13)
6	10.1±0.4(8)	9.5±0.3(10)

The fact that both total protein and blood hemoglobin levels were relatively stable implies that little change in plasma volume occurred in either species. Thus, the changes in the other blood parameters are probably functional rather than due to increased water influx through the damaged gill epithelium.

In conclusion, the juvenile steelhead trout is under a more severe physiological stress during 200 ppm formalin treatments at 10 C than is the spring chinook salmon. However, in neither fish are the histological and physiological disturbances as great as those caused by formalin treatments of rainbow trout (Wedemeyer 1971). In neither the chinook nor steelhead is the stress of 1-hr treatments great enough to cause significant interrenal ascorbate depletion, but blood levels of the "stress hormone" cortisol begin to rise in the steelhead during treatments longer than an hour. Similarly, although gill histological changes are seen, the acid-base balance is adequately compensated and total plasma osmolality is not appreciably disturbed during a 1-hr exposure. However, Cl^- regulation is somewhat compromised in both fishes and, in the steelhead, remains so for up to 24 hr. Thus, it would probably be wise to avoid any fish-cultural operations which would impose additional stress during this recovery period. These would include pond cleaning and handling or stocking immediately following formalin treatments. Even though the fish may seem not to

be harmed by such practices because of their remaining homeostatic capabilities, these should not be taken as an excuse for subjecting them to unnecessary stress.

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Annual Biodeposition and Gross Energy Budget of Mature Pacific Oysters, *Crassostrea gigas*

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BERNARD, F. R. 1974. Annual biodeposition and gross energy budget of mature Pacific oysters, *Crassostrea gigas*. J. Fish. Res. Board Can. 31: 185–190.

The mean annual volume of seawater pumped by Pacific oysters in Departure Bay, British Columbia is 1488 liters/g per year. The energy requirement for metabolism and pumping is 1.74 cal/liter, while the mean annual caloric content of the seawater is 60.4 cals/liter. Annual biodeposition is 8.9 g/g per year; these data applied to a mean biomass of 190 g/m² for a mature oyster reef show that 22,052 kcal are theoretically available for ingestion, but only 11% are retained, mostly to be biodeposited. The biodeposits have a caloric value of 1545 kcal/m² and furnish an important nutritional substrate. Another important contribution to the food web is the annual release of gametes, equivalent to 502 kcal/m² reef.

BERNARD, F. R. 1974. Annual biodeposition and gross energy budget of mature Pacific oysters, *Crassostrea gigas*. J. Fish. Res. Board Can. 31: 185–190.

Le volume annuel moyen d'eau de mer pompée par les huîtres du Pacifique de la baie Departure, Colombie-Britannique est de 1488 litres/g par an. L'énergie requise pour le métabolisme et le pompage est de 1.74 cal/litre, alors que le contenu calorique annuel moyen de l'eau de mer est de 60.4 cal/litre. La biodéposition annuelle est de 8.9 g/g par an; ces données, appliquées à une biomasse moyenne de 190 g/m² pour un récif d'huîtres matures, démontrent qu'en théorie 22,052 kcal sont disponibles pour l'ingestion, mais que seulement 11% sont conservées, pour être en grande partie biodéposées. La valeur calorique des biodépôts est de 1545 kcal/m², et ils constituent un substrat nutritif important. Une autre importante contribution au réseau alimentaire est la libération annuelle de gamètes, équivalent à 502 kcal/m² de récif.

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PACIFIC oysters, indigenous to Japan, do not normally reproduce in significant numbers in British Columbia, except in a few favored locations with high water temperatures in summer. In 1958 there occurred a widespread breeding on commercial plantings and the entire southern portion of the Strait of Georgia was populated, resulting in the establishment of oyster reefs with a dominant year-class that displayed little somatic growth subsequent to 1963. These reefs undergo a cyclic accumulation of reserves, gametogenesis, and release of gametes to the environment. The reefs are important consolidators and sedimenters of suspended materials, and by their biodepositional activities have profoundly modified regions that previously did not support large filter-feeding populations.

This study monitors for 1 yr the biodeposition of a representative sample of oysters collected from the Ladysmith Harbour reef. Determinations of pumping and respirational rates are combined with caloric and gross constituent analyses of the deposits and particulate contents of the waters. An attempt is made to relate the energy flux through a square meter of reef to the food contents of overlying waters, and to indicate the trophic significance of the biodeposits.

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Materials and Methods

Twelve mature oysters, representative of the Ladysmith reef, were held for 1 yr in individual dishes placed in a deep rectangular tray on a float provided with surface water pumped from Departure Bay by two submersible pumps. Care was taken to supply more water than theoretically passes through the pallium (F. R. Bernard unpublished data). The weight of the oysters in air and water, and their total volumes, were recorded weekly. Ten similar oysters held under identical conditions were sacrificed each month and the soft parts weighed. A conversion factor, obtained by dividing the meat weight by the total weight in water, was applied to the experimental oysters to calculate the tissue weight.

Twice weekly the tray was drained and deposits collected with a suction apparatus, no attempt being made to segregate fecal from pseudofecal material. The collected deposits were concentrated by centrifuging, then freeze-dried. The inorganic components were estimated by ignition for 1 h at 600 C. Caloric values were estimated by bomb calorimetry with the Phillipson (1964) modification for small samples; total crude protein by the Kjeldahl-Gunning method for nitrogen, converted to protein using the standard nitrogen/protein factor (Giese 1967); fats by the diethyl ether extraction technique; and carbohydrates by the classical anthrone reaction, calibrated against a glucose standard. The protein, fat, and caloric determinations were undertaken by a commercial laboratory, and the carbohydrate values determined by the author.

Physiological and morphological data were obtained from a large number of oysters collected from the Ladysmith reef and maintained under conditions similar to the raft-held individuals. The mean pumping rates of these oysters at various temperatures were established by direct collection of water using a modification of the Galtsoff (1926) constant-level tank and rubber dam (Moore 1910). Oxygen consumption under various pumping loads was determined using the Collier (1959) modification of the Krogh (1935) technique.

To standardize the body weight as a function of shell volume, a correction derived from Quayle's (1969) regression was applied, where C is the condition factor,

$$\text{Weight} = 0.346 \text{ vol} + 0.112 \times \text{c-26}$$

In this study a condition factor of 50 is utilized; this represents a thin oyster where the pallial organs in proportion to the body are at their greatest.

As it is not possible to measure directly the release of gametes into the environment, or to obtain complete spawning of tank-held oysters, estimations of gamete production were based upon the dissecting away of reproductive tissues from fully ripe individuals and weighing after centrifuging to remove excess water.

Records of the weight of suspended materials in the sea water supplied to the experimental oysters were obtained twice weekly using a Gelman 0.45 μ pore-size glass-filter technique outlined in Strickland and Parsons (1968). The organic content of the suspension was estimated by wet oxidation of carbon by acid dichromate (Strickland and Parsons 1968). This oxidizable carbon value is a rough measure of energy potential, and total organic matter may be calculated assuming a 5% relationship.

Results

The total monthly weights of the soft parts of the control group and the calculated weights of the experimental oysters, with the conversion factors, are given in Table 1. The last column of Table 1 lists the mean tissue weight per square meter of the Ladysmith reef, calculated from four replicate samples taken four times over the year of study. The annual mean was 1562 g/m^2 wet weight (234 g/m^2 dry weight).

Oyster tissues collected throughout the year and subjected to bomb calorimetry ranged between 4877 to 5145 cal/g (dry weight), with an annual mean of 5003 cal/g. The average caloric contents for mixed gametes was 4786 cal/g (dry weight). Large individuals produced proportionately more gametes than small oysters; the mean proportion of gonadal tissues in the largest individuals was over 50%, in comparison with only 32% in the smallest, the mean for the Ladysmith reef being 45.5% of total meat weight just prior to spawning.

The total monthly biodeposit production, with inorganic percentage and caloric values are presented in Table 2, and percentage composition of protein, fats, and carbohydrates, are graphed in Fig. 1. The monthly mean records of suspended material present in the natural sea water presented to the experimental and control oysters are given in Table 3, with the estimated total weight of organic material and its caloric value.

The mean pumping rates for three oysters in each of five experiments at each temperature and the oxygen consumptions (determined separately) are given in Table 4.

The mean monthly weight of the soft parts, coupled to the measured temperatures, permit the estimation of the volume of water pumped through the pallial cavity. These values applied to the known load of suspended material present will approximate

TABLE 1. Monthly biomass of Ladysmith reef and tissue weight of 10 control group oysters, with conversion factor applied to calculate weight of 12 tray-held oysters, July 1971–June 1972.

Month	Wt. of control group (g)	Factor	Wt. of experimental group (g)	Biomass (g/m^2)
July	820	0.270	662	1760
Aug.	523	0.243	555	
Sept.	922	0.321	728	1814
Oct.	830	0.254	591	
Nov.	800	0.243	567	
Dec.	698	0.241	576	
Jan.	673	0.240	576	1230
Feb.	542	0.243	567	
Mar.	510	0.221	569	
Apr.	573	0.214	503	1446
May	556	0.253	503	
June	771	0.280	662	
$\overline{X}$	684		588	1562

the quantity of particles susceptible to retention, here termed the "available load." In Table 5 the calculated monthly available loads are given with the percentage efficiency of retention, calculated from the biodeposit weights (Table 2). The retention efficiency varies between 7.5 to 37.1%, with an annual mean of 23.6% and an overall efficiency of 16.6%.

Using Jorgensen's (1955) formula relating work to volume pumped and assuming a 7 mm hydrostatic head (F. R. Bernard unpublished data), each liter of water pumped requires 6.867×10^5 ergs. Accepting that 1 cal is equal to 4.186×10^7 ergs, each liter pumped consumes 0.0164 cal. At 15 C the Pacific oyster consumes 0.012 ml O_2 while pumping 34.4 cm^3 (Table 4), so that 1 ml O_2 is used for every 2.87 liters pumped. It follows that 1.971×10^6 ergs are expended on water pumping for every 4.186×10^7 ergs released. This pumping expenditure is only 4.7% of the gross, an unexpectedly low proportion, but agrees with that calculated by Jorgensen (1955), though he used a greater pumping volume in his determination.

If 2.87 liters of water are pumped for 1 ml O_2 consumed, and accepting an oxy-caloric coefficient

TABLE 2. Total monthly biodeposition by 12 tray-held oysters with organic percentage and caloric values, July 1971–June 1972.

Date	Total (*g*)	% organic	Caloric value (*cal*)
July	83.2	33.8	54,729
Aug.	68.8	31.6	66,546
Sept.	79.6	30.0	65,070
Oct.	50.7	27.8	29,185
Nov.	77.8	24.4	26,313
Dec.	54.2	21.9	17,704
Jan.	47.7	18.8	12,048
Feb.	39.4	18.6	20,987
Mar.	55.7	20.2	49,621
Apr.	59.9	27.7	74,487
May	82.5	41.2	150,039
June	73.8	46.5	143,341
Total	773.3		710,670

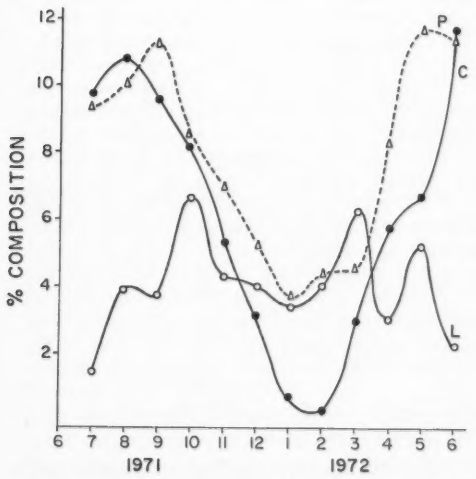

FIG. 1. Monthly percentages of proteins (P), carbohydrates (C), and lipids (L) in oyster biodeposits.

TABLE 3. Concentration of suspended matter in Departure Bay sea water with organic contents and caloric values, June 1971–July 1972.

Month	Particulate matter (*mg/liter*)	Organic (*mg/liter*)[a]	Caloric value (*cal*)[b]
July	41.6	18.2	72.8
Aug.	19.8	9.4	37.6
Sept.	21.3	4.3	17.2
Oct.	38.9	15.0	60.0
Nov.	36.2	14.2	56.8
Dec.	42.4	12.3	49.2
Jan.	46.0	9.7	38.8
Feb.	47.5	7.2	28.8
Mar.	40.6	16.2	64.8
Apr.	42.6	10.0	40.0
May	47.4	26.6	106.4
June	46.3	39.2	156.8
$\bar{X}$	39.2	15.1	60.7

[a]Calculated from carbon values.
[b]Estimated using 4000 cal/g carbon.

TABLE 4. Mean volume pumped and oxygen consumption per gram tissue wet weight at various temperatures, averaged from 500 oysters.

Temp (*C*)	Vol pumped (*liter* × *10*$^{-2}$*/g/h*)	Oxygen utilization (*ml* O_2*/g/h*)
5	0.67	0.002
10	1.65	0.008
15	3.44	0.012
20	6.90	0.022
25	7.68	0.026
$\bar{X}$	4.068	0.014

TABLE 5. Theoretical availability of suspended materials and volume pumped compared to total monthly biodeposition by 12 tray-held oysters, July 1971–June 1972.

Month	Volume (*liters*)	Load (*g*)	Deposited (*g*)	Retention (%)
July	24,829	1,103	83.2	7.5
Aug.	26,514	516	68.8	13.3
Sept.	15,874	341	79.6	23.3
Oct.	9,355	329	50.7	15.4
Nov.	6,280	235	77.8	33.1
Dec.	3,721	160	54.2	33.8
Jan.	2,954	134	47.7	35.6
Feb.	2,951	140	39.4	28.1
Mar.	3,772	150	55.7	37.1
Apr.	4,334	185	59.9	32.4
May	13,117	633	82.5	13.0
June	15,804	732	73.8	10.0
Total	129,505	4,658	773.3	$\bar{X}$ 23.6

of 5 cal/ml O_2 (Weinberg 1956; Paloheimo and Dickie 1966), then 1 liter of water is pumped using 1.74 cal. If 1.74 cal are required for each liter pumped and the maintenance of other physiological activity, it follows that each liter of sea water must on average contain at least this energy value if the organism is not to suffer a net loss. The average organic contents of Departure Bay sea water over a 1-yr period was 15.1 mg/liter (Table 3), equivalent to approximately 60.4 cal. The lowest recorded suspended value, obtained in September, provided only 2 cal/liter. This is not sufficient, and utilization of reserves would occur, though dissolved organic compounds may contribute in this situation.

The Pacific oyster is able to withstand prolonged periods of inanition and may be kept many months in filtered sea water with very little decrease in condition factor. Converting the 20 C oxygen consumption value given in Table 4 to dry weight (0.1466 ml O_2/g per hour) and accepting the oxycaloric coefficient of 5 cal/ml O_2, 1 g of dry tissue consumes 0.733 cal/h. As 1 g dry weight tissue is equivalent to 5003 cal, 1 g of tissue will support full pumping activity at 20 C for 284 days. This converts to 43 days/g tissue wet weight. Utilization of reserves is greatly reduced at lower temperatures, 1 g tissue being equivalent to 120 days activity at 10 C.

The biomass determinations for the Ladysmith reef (Table 1) applied to the pumping and oxygen consumption levels established (Table 4), permit an estimation of total energy flux through 1 m² of oyster reef, as follows:

Biomass (dry weight)	234 g
Biomass (condition factor 50)	190 g
Biomass caloric value	1170 kcal
Gamete production	105 g
Gamete caloric value	502 kcal
Total volume pumped	282,720 liters
Caloric cost of pumping	503 kcal
Calories available in water	22,052 kcal
Biodeposits (total)	1691 g
Biodeposits (organic)	551 g
Biodeposits (caloric value)	1545 kcal

Assuming 70% of the time is spent pumping and the reef is permanently submerged, a schematic representation of caloric distribution for a 1-yr period is given in Fig. 2. Somatic accumulation is not included, and is probably marginal, but the annual return of gametes to the environment is shown. Another biomass debit is the result of excretion. Little is known about the process in bivalves, but Hammen et al. (1966) showed that *C. virginica* is ammonotelic, in which case only slight caloric loss occurs. There is also a probable further loss due to amino acid leakage to the environment (Hammen 1968). A portion of the ingested food goes to build the shell which represents a significant energy pool, comprising approximately 2% by weight of organic matrix and hinge ligament.

Discussion

Suspension feeders serve as an important and direct link between suspended organic particles

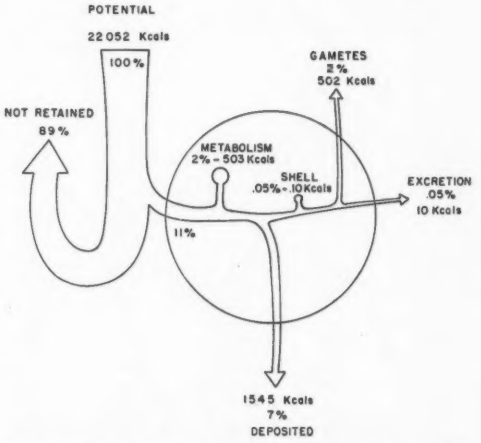

FIG. 2. Schematic representation of percentage distribution of potential food expressed in kilocalories for a 1-yr period in 1 m² of mature oyster reef.

and a wide assemblage of benthic populations, including polychaetes, holothurians, and crustaceans, Production of biodeposits calculated in this study are 2082 g/m^2 per year, while in the same area, Stephens (1966) and Stephens et al. (1967) recorded an annual gravitational deposition of 2151 g/m^2 for a 45-m water column, but particle size was larger and inorganic contents higher. Haven and Morales-Alamo (1966) have shown that bivalves initiate deposition of particles in the 1–3 μ range and consolidate suspended matter faster than detrital sedimentation alone. Detrital sediments include fecal pellets from planktonic and pelagic organisms, for instance Moore (1931) reported that *Calanus* and euphausiids in the spring deposited the equivalent of 47 g/m^2 per day in the Clyde area. Oysters are instrumental in depositing material equal to that resulting from the combined activity of plankton and gravitational sedimentation in a 45-m column of water, and the biodeposited particles are smaller and have a higher nutritive potential to browsing organisms.

These large quantities of biodeposits, besides providing a nutritional source for other forms, also modify the physical and chemical characteristics of the substrate sediments, allowing the establishment of diverse groups. The deposits may also serve as substrate for bacteria and other small organisms that may ultimately participate in oyster nutrition, as sedimented aggregates are readily resuspended by water movements and may be nutritionally important. In this way oyster reefs may alter their niche and increase productivity.

The large volumes of nutritional deposits rejected by the ctenidial apparatus, or passing undigested through the alimentary canal, suggest that Pacific oysters are either inefficient or highly selective in their digestive ability. Miller (1967) suggested that a possible specialization of suspension feeders may prevent competitive exclusion due to identical feeding habits. This presumes that unused particles are available to other suspension feeders, but indications are that the majority of particles in the inhalant stream are sedimented and not available directly to other suspension feeders. The apparent inefficiency of retention shown in this study is probably attributable to the reef community of oysters, leading to consecutive filtering of the same water mass, and strongly suggests that, next to temperature, adequate water exchange is the prime factor in oyster growth.

In small oysters somatic growth is the major energy assimilator, and growth data (Quayle 1969) show that in the Pacific oyster growth is exceptional for the first 4 yr; it then decreases, with large oysters merely going through a cycle of gametogenesis. This study shows that, prior to breeding, nearly half the total biomass of the oyster reef consists of gametes, which are either reabsorbed or released, depending upon the temperature regime. Gamete release may be an important factor in planktonic productivity, as gametes and resulting larvae are released after the period of maximum phytoplanktonic production is over, and may furnish an important food supply for crustacean and other planktonic carnivores. The amounts involved are substantial, at least 105 g/m^2 of natural reef, with a caloric value in excess of 500 kcal.

The difference between the theoretical energy expenditure required for pumping and the observed oxygen consumption is great, and it is difficult to accept that 95% of liberated energy is utilized for processes other than pumping. These calculations are based upon minimum requirements in a frictionless system with perfect energy transfer. However, Ludwig (1931) estimated only 2% efficiency for individual ciliated cells, so oxygen requirements may be high in relation to work done. Further losses in efficiency are probably due to friction in passage through the pallial cavities, small ostia, and particularly the water tubes where a large angular change of flow direction occurs. The actual energy requirement for pumping is probably 10 or more times the theoretical value.

Oyster biomass production dependent upon in situ primary productivity can average no more than 15 g/m^2 per year (Ryther 1969), yet the Ladysmith reef yielded an average 105 g/m^2 per year of gametes, showing that nutrition is drawn from a wide productive source. It is this concentration of particulate material that has an influence on the ecosystem far in excess of the actual biomass. By far the largest part of retained particles is channeled into biodeposits and these large quantities of sedimented materials play an important role in trophic relationships, supporting a wide assemblage of small invertebrates within the reef itself, or exported by water movements and raising the general productivity of adjacent zones.

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Residues of DDT in Lake Trout (*Salvelinus namaycush*) and Coho Salmon (*Oncorhynchus kisutch*) from the Great Lakes[1]

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Concentrations of DDT residues were higher in lake trout (*Salvelinus namaycush*) from southern Lake Michigan in 1966–70 (average 18.1 ppm in fish 558–684 mm long) than in lake trout of the same size-class from Lake Superior in 1968–69 (4.4 ppm), and higher in adult coho salmon (*Oncorhynchus kisutch*) from Lake Michigan in 1968–71 (averages for different year-classes, 9.9–14.0 ppm) than in those from Lake Erie in 1969 (2.2 ppm). Residues were significantly higher in lake trout from southern Lake Michigan than in those from the northern part of the lake. In lakes Michigan and Superior, the levels increased with length of fish and percentage oil. In Lake Michigan coho salmon, the residues remained nearly stable (2–4 ppm) from September of the 1st yr of lake residence through May or early June of the 2nd yr, but increased three to four times in the next 3 mo. Residues in Lake Erie coho salmon did not increase during this period, which preceded the spawning season. Although the concentrations of total residues in whole, maturing Lake Michigan coho salmon remained unchanged from August 1968 until near the end of the spawning season in January 1969, the residues were redistributed in the tissues of the spawning-run fish; concentrations in the loin and brain were markedly higher in January than in August. This relocation of DDT residues accompanied a marked decrease in the percentage of oil in the fish, from 13.2 in August to 2.8 in January. Concentrations of residues were relatively high in eggs of both lake trout (4.6 ppm) and coho salmon (7.4–10.2 ppm) from Lake Michigan. The percentage composition of the residues (*p,p'*DDE, *o,p'*DDT, *p,p'*DDD, and *p,p'*DDT) did not differ significantly with life stage, size, age, or locality, or date of collection of lake trout or coho salmon.

REINERT, R. E., AND H. L. BERGMAN. 1974. Residues of DDT in lake trout (*Salvelinus namaycush*) and coho salmon (*Oncorhynchus kisutch*) from the Great Lakes. J. Fish. Res. Board Can. 31: 191–199.

La teneur en DDT des touladis (*Salvelinus namaycush*) du secteur méridional du lac Michigan est plus élevée (18.1 ppm en moyenne chez des poissons de 558–684 mm de longueur) que celle des touladis de même taille du lac Supérieur (4.4 ppm). Elle est plus élevée chez les saumons coho adultes (*Oncorhynchus kisutch*) du lac Michigan (moyennes de différentes classes d'âge, 9.9–14.0 ppm) que chez ceux du lac Erié (2.2 ppm). Les résidus sont nettement plus élevés chez les touladis du lac Michigan méridional que chez ceux de la partie nord du lac. Dans les lacs Michigan et Supérieur, les niveaux augmentent avec la longueur du poisson et le pourcentage d'huile. Chez les saumons coho du lac Michigan, les résidus de DDT demeurent presque stables (2–4 ppm) entre septembre de la 1ere année de résidence dans le lac et mai et juin de la 2e année, mais augmentent de trois ou quatre fois au cours des 3 mois qui suivent. Les résidus, chez les saumons coho du lac Erié, n'augmentent pas durant cette période qui précède la saison de fraie. Bien que les teneurs en résidus totaux chez les saumons coho entiers en voie de maturation du lac Michigan n'aient pas changé entre août 1968 et la fin de la fraie en janvier 1969, les résidus

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s'étaient redistribués dans les tissus des poissons de la montaison de fraie; les concentrations dans le rein et le cerveau étaient notablement plus élevées en janvier qu'en août. Cette redistribution des résidus de DDT est accompagnée d'un déclin marqué du pourcentage d'huile dans le poisson, de 13.2 en août à 2.8 en janvier. Les concentrations de résidus sont relativement élevées dans les oeufs, tant du touladi (4.6 ppm) que du saumon coho (7.4–10.2 ppm) du lac Michigan. La proportion des résidus (*p,p'*DDE, *o,p'*DDT, *p,p'*DDD et *p,p'*DDT) ne diffère pas de façon significative avec le stade du cycle biologique, la taille, l'âge, l'endroit ou la date de capture, ni chez le touiadi ni chez le saumon coho.

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ALTHOUGH general information concerning DDT residues in Great Lakes fish is available (Carr et al. 1972; Henderson et al. 1971; Poff and Degurse 1970; and Lake Michigan Interstate Pesticides Committee of the Lake Michigan Enforcement Conference 1972), there are few data concerning the concentrations of these residues accumulated at different life stages of specific species. In the present study, we determined concentrations of DDT residues in lake trout of various lengths from lakes Michigan and Superior; in coho salmon of various lengths from Lakes Michigan and Erie; and in eggs and fry of lake trout and eggs of coho salmon from Lake Michigan. We also compared concentrations of DDT residues and the percentages of oil in adult coho salmon (whole fish, steaks, portions of steaks, and brains) from Lake Michigan with those collected from the Platte River during their spawning migration.

As each year-class of lake trout stocked (as yearlings) in Lake Michigan since the mid-1960s was distinctively marked with a specific fin clip, and since the life of coho salmon is short (about 15–17 mo each in the hatchery and in the lake), it was relatively easy to identify the year-classes of both species throughout their life cycles.

Materials and Methods

Lake trout and coho salmon were collected for analysis by the Great Lakes Fishery Laboratory, the Michigan Department of Natural Resources, and the Wisconsin Department of Natural Resources. Fish were frozen as soon as possible after capture and stored at the Great Lakes Fishery Laboratory at –36.7 C.

Lake trout adults, eggs, and fry and coho salmon and coho salmon eggs were sampled for DDT residues and oil. Lake trout were sampled annually in 1966–70 in each of four regions of Lake Michigan (northeast, northwest, southeast, and southwest) and in 1968–69 in State of Michigan waters of Lake Superior. Concentrations of DDT residues in adult lake trout in the four regions of Lake Michigan were compared (Wilcoxon matched-pairs, signed-ranks test; 95% confidence level). Eggs taken in November 1970 from 10 spawning-run lake trout of the 1965 year-class in northern Lake Michigan were hatched at the Jordan River National Fish Hatchery. Samples of 2–5 g (about 11 eggs/g) were analyzed from each fish.

Coho salmon of the 1965 year-class were sampled periodically during each of their two growing seasons in Lake Michigan. Collections were made in the southern half of the lake from September 1967 through June 1968; in the northern half in July and August 1968; and at a weir in the Platte River (a tributary of northern Lake Michigan), 16.1 km (10 miles) upstream from the lake, in October 1968 and January 1969. Eggs were collected from these fish in August and October 1968 and January 1969. Fish from the 1966 to 1968 year-classes were sampled during their 2nd yr in the lake, in southern Lake Michigan in the spring and in the northern part of the lake in late summer. In Lake Erie, fish from the 1966 year-class were sampled in the spring and fall of their 2nd yr in the lake.

To determine changes in concentrations of DDT residues in various tissues of coho salmon during the spawning season, we measured DDT and oil content in a whole, 25-mm thick steak (cut just anterior to the dorsal fin) and different parts of a steak, and the brain, eggs, and the remainder of fish taken before and during the spawning season.

Before analysis, whole fish or selected portions were partly thawed and ground three times in one of several sizes of Hobart meat grinders. After the ground sample was thoroughly mixed, two 10-g wet weight portions were analyzed (for the analysis of specific tissues, it was sometimes necessary to use amounts smaller than 10 g). One 10-g sample was saponified in alcoholic KOH and extracted with hexane; the other was homogenized in a 1:1 mixture of isopropanol and benzene and extracted with hexane. Residues (*p,p'*DDE, *o,p'*DDT, *p,p'*DDD, and *p,p'*DDT) were analyzed on a model 204 Aerograph gas chromatograph equipped with two electron capture detectors. Extraction procedures, analytical procedures, and operating conditions of the gas chromatograph were described by Reinert (1970). Concentrations are expressed as parts per million (micrograms of residue per gram wet weight of tissue) in whole fish, steaks, portions of steaks, and brains. All samples were analyzed at the Great Lakes Fishery Laboratory.

The concentrations of oil in fish (whole fish and selected portions) were determined by transferring 15 ml of hexane extract from the samples homogenized in isopropanol and benzene to a tared aluminum dish, and evaporating the solvent in a hood at room temperature; the sample was then weighed and the percentage oil calculated.

To determine if polychlorinated biphenyls (PCB's) were causing a significant error in the analysis of the DDT residues, we analyzed samples of 14 whole lake trout and 10 whole coho salmon from Lake Michigan for DDT residues, and the Fish-Pesticide Research Laboratory of the Bureau of Sport Fisheries and Wildlife, Columbia, Mo., analyzed samples from the same fish for DDT residues and PCB's. The average concentrations of DDT residues were as follows (± 95% confidence interval in parentheses):

Laboratory	*Lake trout*	*Coho salmon*
Great Lakes	11.1(1.6)	8.5(1.0)
Fish-Pesticide	8.7(1.5)	10.4(3.1)

The average concentrations of PCB's were 16.5 ppm in lake trout and 16.4 ppm in coho salmon. These data suggest that, although the concentrations of PCB's in lake trout and coho salmon from Lake Michigan were high, these compounds did not cause significant errors in our analyses of DDT residues.

Residues in Lake Trout

Concentrations of DDT residues increased steadily with length and percentage oil of lake trout from Lakes Michigan and Superior (Table 1). In Lake Michigan, concentrations were significantly higher ($P < 0.05$) in fish from the southern part of the lake than in those of similar size-groups from the northern part; for fish of the smallest and largest length-classes represented (50–150 mm and 558–684 mm), the concentrations were 1.3 and 18.1 ppm, respectively, in the south and 0.8 and 12.9 ppm in the north. Within the southern and northern regions, the concentrations in fish from eastern and western Lake Michigan (data not shown separately in Table 1) did not differ significantly. Percentage oil varied little in fish of identical length-classes from different quadrants of the lake.

Concentrations were markedly lower in lake trout from Lake Superior than in those from Lake

TABLE 1. Average concentrations (ppm) of DDT residues and percentage oil in lake trout (*Salvelinus namaycush*) of different length-groups collected in northern and southern Lake Michigan in 1966–70 and in Michigan waters of Lake Superior in 1968–69 (95% confidence intervals are shown in parentheses).

Region and length (*mm*)	No. of fish	*p,p'* DDE	*o,p'* DDT	*p,p'* DDD	*p,p'* DDT	Total residues	Oil (%)
			Lake Michigan				
Northern region[a]							
50–150	18	0.4(0.2)	0.1(0.0)	0.1(0.0)	0.2(0.0)	0.8(0.2)	3.7(1.9)
151–252	35	0.9(0.2)	0.3(0.1)	0.3(0.1)	0.6(0.3)	1.9(0.5)	8.4(1.9)
253–404	31	1.5(0.9)	0.2(0.2)	0.2(0.2)	0.8(0.2)	2.6(0.4)	11.0(3.0)
405–557	26	2.7(0.5)	0.6(0.2)	0.6(0.1)	2.1(0.4)	6.1(1.2)	17.4(1.4)
558–684	28	6.7(1.3)	1.5(0.4)	1.0(0.2)	3.5(0.6)	12.9(2.3)	21.8(1.5)
Southern region[a]							
50–150	3	0.5(0.5)	0.1(0.2)	0.2(0.3)	0.4(0.4)	1.3(0.7)	4.2(12.2)
151–252	63	0.7(0.2)	0.2(0.0)	0.6(0.2)	1.0(0.2)	2.6(0.6)	7.3(1.6)
253–404	45	1.6(0.3)	0.6(0.2)	0.9(0.2)	2.1(0.3)	5.2(0.8)	10.2(1.1)
405–557	37	3.5(1.2)	1.2(0.2)	1.2(0.2)	3.9(0.6)	9.7(1.3)	19.2(1.7)
558–684	14	8.6(1.3)	2.4(0.8)	1.7(0.2)	5.3(0.7)	18.1(2.5)	21.0(2.2)
			Lake Superior				
Michigan waters[b]							
253–404	13	0.6(0.2)	0.1(0.0)	0.1(0.0)	0.3(0.0)	1.0(0.2)	5.5(1.6)
405–557	10	0.7(0.4)	0.2(0.1)	0.1(0.0)	0.5(0.1)	1.4(0.3)	12.8(3.2)
558–684	9	2.3(0.7)	0.6(0.2)	0.2(0.1)	1.3(0.2)	4.4(0.6)	19.0(3.3)
685–810	31	3.2(0.6)	0.9(0.2)	0.3(0.1)	1.9(0.2)	6.4(0.5)	17.0(1.1)

[a]The northern and southern regions of Lake Michigan were divided by a line extending from Ludington, Mich., to Kewaunee, Wis.

[b]The concentrations of DDT residues, as well as oil content, were much higher in siscowets (oily, deep-bodied lake trout sometimes classified as a subspecies, *Salvelinus n. siscowet*) collected in Michigan waters of Lake Superior in 1969 than in the "lean" lake trout for which data are shown here. In six siscowets 615–725 mm long, average DDT residue concentration was 11.7 ppm and oil content 45.1%.

Michigan (Table 1). The average in the largest size-class of Lake Superior fish sampled (685–810 mm) was 6.4 ppm, and in fish of length-groups 253–404 to 558–684 mm it was only 14–24% of that in fish from southern Lake Michigan. The percentage of oil in lake trout from Lake Superior was also generally lower than that in lake trout of similar size from Lake Michigan.

Concentrations of DDT residues were much higher in adult lake trout than in eggs and fry. For fish of the 1965 year-class collected in southern Lake Michigan in 1970, average concentrations were 17.8 ppm in ripe females, 4.6 ppm in eggs from these fish, and 2.6 ppm in fry hatched from the eggs (Table 2).

The percentage composition of residues in lake trout did not differ significantly with locality or with life stage or length of fish (Table 1 and 2). The predominant forms were *p,p'*DDE and *p,p'* DDT; *o,p'*DDT and *p,p'*DDD made up much smaller proportions of the total.

Residues in Coho Salmon

Coho salmon in Lake Michigan apparently accumulated by far the most DDT in the June–August period preceding the beginning of the spawning migration (Table 3). Fish from the 1965 year-class contained 4 ppm or less of DDT residues from September of their first growing season through early June of their second; by the following August, however, concentrations had more than tripled, to 12.3 ppm. Fish from 1966 to 1968 year-classes, sampled in spring and late summer of their second growing season, showed a similar rapid increase in residues. In each of the four year-classes, fish weight and percentage oil also increased rapidly during the summer preceding the spawning season.

Coho salmon of the 1966 year-class collected in 1969 in Lake Erie did not show the summer increase in DDT residues observed in maturing coho salmon from Lake Michigan; concentrations in the Lake Erie fish were 1.3 and 2.1 ppm in April and May and 2.2 ppm in October (Table 3). Carr et al. (1972) also presented data showing that concentrations of DDT residues in coho salmon in Lake Erie did not increase appreciably during the summer preceding the spawning season. We suggest two possible reasons for this difference between salmon from the two lakes: (1) the concentrations of DDT residues in forage species were substantially lower in Lake Erie than in Lake Michigan (Reinert 1970), and (2) the food intake of salmon was probably lower in Lake Erie than in Lake Michigan, as the average weight of Lake Erie salmon in the fall preceding the spawning season was only about half that of Lake Michigan fish.

Although concentrations and total DDT residues in whole Lake Michigan coho salmon collected during their spawning run (in the Platte River) in October 1968 and January 1969 differed little from those in fish collected from the lake in August 1968 (12.3–12.6 ppm and 43,222–46,371 µg), the location of the residues within the fish changed considerably during this period. In October the concentration and total in the brain had doubled (from 1.2 ppm and 0.9 µg to 2.4 ppm and 2.0 µg); and concentrations in the oily dorsal, medial, and ventral portions of steaks (Fig. 1) had become significantly higher ($P < 0.05$) than in August (Table 4). In January the concentration and total in the brain had again doubled (to 5.0 ppm and 4.4 µg), but concentrations in the three portions of steaks had decreased to August levels. Total residues in these portions (which could not be calculated because the parts of the steak were not clearly distinguishable — samples were taken near the center of each part) must have been lower than they were in August, however, because the portions had become visibly smaller as the season progressed. In the loin portions of steaks, concentrations were about twice as high in fish sampled in January (10.1 ppm) as in fish sampled in August (5.6 ppm) and October (4.7 ppm).

The percentage of oil decreased from August to January in all parts of the fish examined except

TABLE 2. Average concentrations of DDT residues (ppm) and percentage oil content in 10 whole ripe female trout collected from southern Lake Michigan in 1970 (average length 672 mm; range 620–760 mm), eggs from all the fish, and fry hatched from the eggs at the Jordan River National Hatchery, Elmira, Mich. (95% confidence intervals are shown in parentheses).

Life stage	*p,p'* DDE	*o,p'* DDT	*p,p'* DDD	*p,p'* DDT	Total residues	Oil (%)
Parents	9.6(1.8)	1.6(0.3)	2.2(0.6)	4.4(0.9)	17.8(3.1)	20.5(3.4)
Eggs	2.5(0.5)	0.4(0.2)	0.4(0.1)	1.3(0.2)	4.6(0.7)	16.8(3.9)
Fry	1.5(0.3)	0.2(0.0)	0.2(0.0)	0.7(0.1)	2.6(0.4)	5.7(0.4)

the brain, where it did not change significantly (Table 4). The percentages in August and January were 13.2 and 2.8, respectively, in whole fish; 67.0 and 8.5 in the ventral portion of steaks; 5.4 and 1.7 in the loin; and 8.7 and 7.7 in the brain. Adipose fat, which contained the highest percentage oil (85.6), was present only in fish collected in August.

Changes in distribution of DDT residues during the spawning migration appeared to be closely related to the general decrease in the amounts of oil. The increased concentrations in the three portions of steaks from August to October were probably due to a combination of two factors: (1) DDT residues became progressively more concentrated in these body parts as the total amount of oil in them declined, and (2) when fish from Lake Michigan moved into the Platte River in October, the adipose body fat was rapidly lost; the oily dorsal, medial, and ventral portions of the steak decreased in size, and the high concentrations of DDT residues deposited in these areas were redistributed to the remaining portions of the body. By January, the size and oil content of these dorsal,

TABLE 3. Average weights, and DDT residues (ppm) and oil contents, for whole coho salmon collected on different dates in Lake Michigan and Lake Erie (95% confidence intervals are shown in parentheses).

Year-class and date of collection	No. fish	Weight (g)	Total residues	Oil (%)
		Lake Michigan		
1965 year-class				
Sept. 6–10, 1967	2	–	4.0(5.1)	–
Dec. 13, 1967	3	1049(304)	3.0(3.7)	–
Apr. 21–22, 1968	5	1054(255)	3.3(0.9)	7.4(2.3)
May 4–7, 1968	3	1180(485)	3.5(1.2)	5.3(2.9)
June 2, 1968	4	1997(309)	3.8(1.6)	9.0(7.4)
July 16–29, 1968	8	2775(351)	9.7(1.2)	12.2(1.5)
Aug. 21, 1968[a]	10	3514(470)	12.3(1.5)	13.2(1.3)
Oct. 18, 1968[a,b]	10	3486(470)	12.6(1.5)	7.1(0.7)
Jan. 31, 1969[a,b]	13	3770(364)	12.3(1.1)	2.8(0.5)
1966 year-class				
May 1, 1969	4	1328(140)	3.5(2.3)	9.0(4.4)
Sept. 3–11, 1969	10	3903(678)	11.8(2.7)	11.6(1.6)
1967 year-class				
May 4, 1970	5	967(335)	2.9(0.4)	6.9(1.8)
Aug. 21–28, 1970	13	3663(500)	14.0(1.3)	13.8(1.6)
1968 year-class				
Apr. 17, 1971	12	1029(104)	1.8(0.2)	7.6(0.6)
Aug. 28, 1971	15	4254(367)	9.9(1.4)	13.0(0.8)
		Lake Erie		
1966 year-class				
Apr. 4, 1969	6	811(291)	1.3(0.4)	10.8(3.6)
May 5, 1969	5	1050(378)	2.1(0.3)	10.0(2.7)
Oct. 7–8, 1969[c]	10	2027(405)	2.2(0.3)	5.2(0.8)

[a]Two 25-mm thick steaks and the brain had been removed from these fish (Table 4).
[b]Fish collected during the spawning migration into the Platte River.
[c]Three fish were collected from Crooke Creek and three from Trout Run (tributaries of Lake Erie); four were collected from Lake Erie near Cedar Point, Ohio.

TABLE 4. Average distribution of DDT residues (ppm) and percentage of oil in Lake Michigan coho salmon collected on three dates (Table 3): concentrations in whole fish (fish minus two 25-mm thick steaks and the brain), whole steaks, portions of steaks (Fig. 1), brain, and eggs (95% confidence intervals are shown in parentheses).

Part of fish and month of collection	*p,p'*DDE	*o,p'*DDT	*p,p'*DDD	*p,p'*DDT	Total residues	Oil (%)
Whole fish[a]						
Aug. 21	6.1(0.9)	1.0(0.2)	1.2(0.2)	4.0(0.7)	12.3(1.6)	13.2(1.3)
Oct. 18	6.6(0.9)	0.8(0.2)	1.0(0.2)	4.1(0.5)	12.6(1.3)	7.1(0.7)
Jan. 31	7.0(0.6)	1.0(0.2)	0.9(0.2)	3.4(0.6)	12.3(1.1)	2.8(0.5)
Steaks						
Whole steaks						
Aug. 21	7.5(1.3)	1.3(0.7)	1.2(0.5)	4.9(0.5)	14.9(2.2)	16.2(1.3)
Oct. 18	8.5(1.1)	1.3(0.5)	1.5(0.5)	5.1(0.7)	16.3(2.0)	7.1(1.1)
Jan. 31	10.3(1.3)	1.5(0.4)	1.5(0.2)	5.1(0.6)	18.4(1.5)	2.3(0.6)
Loin						
Aug. 21	2.8(0.7)	0.5(0.2)	0.5(0.2)	1.8(0.2)	5.6(1.1)	5.4(0.9)
Oct. 18	2.6(0.5)	0.3(0.2)	0.3(0.2)	1.4(0.2)	4.7(0.7)	2.3(0.5)
Jan. 31	5.1(0.9)	1.0(0.2)	0.9(0.2)	3.1(0.6)	10.1(1.7)	1.7(0.4)
Dorsal						
Aug. 21	30.5(5.8)	5.9(1.1)	5.6(0.9)	19.1(2.0)	61.1(8.7)	62.1(1.3)
Oct. 18	57.1(11.3)	8.4(3.3)	8.7(2.0)	31.8(4.0)	106.1(19.2)	39.2(6.2)
Jan. 31	31.4(10.8)	4.3(1.9)	5.5(2.6)	18.3(7.3)	59.6(22.8)	8.2(5.0)
Medial						
Aug. 21	21.4(2.9)	3.7(0.2)	3.1(0.5)	12.9(1.1)	41.1(2.9)	44.5(6.2)
Oct. 18	32.2(7.1)	5.4(1.6)	4.7(0.9)	17.5(2.9)	59.8(11.6)	22.4(2.7)
Jan. 31	23.3(4.3)	3.3(0.9)	3.1(0.9)	11.6(1.9)	41.3(7.1)	7.7(3.2)
Ventral						
Aug. 21	31.4(4.0)	6.6(1.6)	6.9(0.9)	21.1(3.3)	66.0(8.0)	67.0(3.6)
Oct. 18	57.7(8.0)	10.5(2.0)	7.9(2.5)	30.3(4.5)	106.4(13.4)	41.5(8.2)
Jan. 31	37.9(14.5)	5.3(2.2)	6.0(2.8)	19.4(7.3)	68.5(26.1)	8.5(4.9)
Adipose fat[b]						
Aug. 21	44.0(8.2)	8.7(2.0)	8.4(1.3)	27.8(4.0)	88.9(10.7)	85.6(4.0)
Brain						
Aug. 21	1.0(0.5)	0.1(0.0)	0.1(0.0)	0.4(0.2)	1.2(0.5)	8.7(1.3)
Oct. 18	1.2(0.5)	0.2(0.2)	0.1(0.0)	0.8(0.2)	2.4(0.7)	5.8(1.8)
Jan. 31	2.8(0.6)	0.6(0.2)	0.3(0.2)	1.4(0.2)	5.0(1.1)	7.7(1.7)
Eggs[c]						
Aug. 21	3.1(0.2)	0.7(0.2)	0.6(0.2)	2.9(0.9)	7.4(0.9)	10.9(2.0)
Oct. 18	6.0(1.8)	0.9(0.2)	0.5(0.4)	2.8(0.7)	10.2(2.9)	8.6(2.5)
Jan. 31	4.3(0.6)	0.7(0.2)	0.6(0.2)	2.4(0.2)	7.9(0.6)	9.4(2.4)

[a]Average weights of whole fish (95% confidence intervals in parentheses) were 3514 g (470) in August, 3486 g (470) in October, and 3770 g (364) in January.
[b]Fish collected in October and January lacked adipose fat.
[c]Eggs were collected from three fish in August, four in October, and eight in January.

medial, and ventral parts had apparently been reduced to the point that some of the insecticide was redistributed to the loin and brain.

Concentrations of DDT residues in eggs of coho salmon of the 1965 year-class collected in Lake Michigan in August 1968 (7.4 ppm, Table 4) were not significantly different from these taken during the spawning migration in October 1968 (10.2 ppm) or January 1969 (7.9 ppm). Concentrations in eggs of coho salmon were significantly higher than those in eggs of lake trout (Table 2).

Proportions of the various residues of DDT in coho salmon (Table 4) were closely similar to those in lake trout (Table 1), and did not vary significantly among the different tissues examined.

Discussion

The rate of accumulation of DDT residues by lake trout and coho salmon in Lake Michigan appeared to be related in part to rate of growth. The relatively slow-growing lake trout accumulated residues at a nearly constant rate, whereas coho salmon from Lake Michigan accumulated most of the insecticide during the period of exceptionally rapid growth preceding the spawning season. This rapid accumulation is probably related, at least in part, to a substantial increase in the intake of DDT-contaminated food (alewives, *Alosa pseudoharengus*, the principal food of lake trout and coho salmon in Lake Michigan, typically contained 1–4 ppm DDT residues — see Reinert 1970). In addition, the large amounts of oil accumulated during the summer preceding spawning may facilitate the storage of DDT residues.

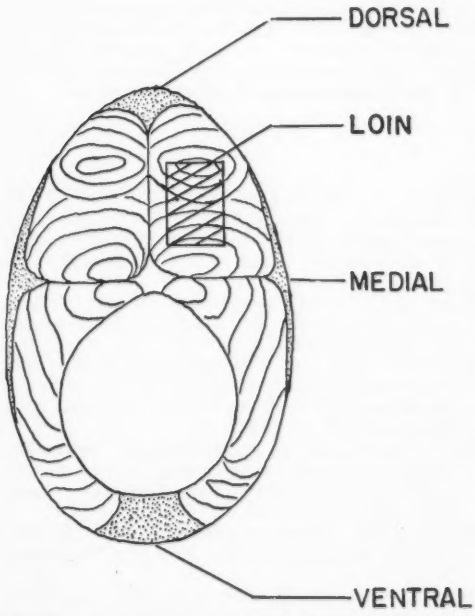

FIG. 1. Portions of coho salmon (*Oncorhynchus kisutch*) examined for DDT residues and oil.

Generally, in Lake Michigan, lake trout larger than 405 mm and coho salmon collected in late summer and fall of their second growing season in the lake contained concentrations of DDT above the 5-ppm interim guideline level of the U.S. Food and Drug Administration (FDA). Although the trimming of fat from the dorsal, medial, and ventral parts of these species could be expected to lower the concentration of DDT residues (Reinert et al. 1972), concentrations in the loin of coho salmon taken near the end of the spawning season were so high (about double the FDA guideline level) that trimming would provide little benefit.

The percentage compositions of DDT residues in lake trout and coho salmon were similar to those found by Henderson et al. (1971) for a number of species of fish from many localities in the United States. This similarity in composition in different species, sizes, and tissues of fish suggests that a substantial part of the degradation of DDT occurs before the insecticide is accumulated by fish. Two probable agents of degradation are aquatic invertebrates (Johnson et al. 1971) and bacteria (Wedemeyer 1966; Guenzi and Beard 1967).

Mortalities of animals that were related to redistribution of DDT residue and increased concentrations of residues in the brain have been reported in other studies. Ecobichon and Saschenbrecker (1969) and Van Velzen et al. (1972) observed that the death of birds in the laboratory following food deprivation was associated with mobilization of oil, redistribution of DDT residues, and increased concentrations of residues in the brain. The observation that a loss of oil by fish results in a redistribution of DDT residues was made in the laboratory by Grant and Schoettger (1972). When rainbow trout (*Salmo gairdneri*) that had accumulated DDT residues were starved and forced to swim in a current for 28 days, there was no net loss of DDT residues; however, body fat decreased progressively throughout the period, the concentration of DDT residues in the brain increased sharply after 15 days, and the concentrations in blood and muscle increased after 20 days.

No mortalities of adult lake trout or coho salmon have been reported from the Great Lakes that can be attributed to the effects of DDT residues. However, the range of concentrations (2.5–8.6 ppm)

in the brains of coho salmon from Lake Michigan, collected in January 1969, slightly overlapped the range (8–16 ppm) that Grant and Schoettger (1972) found in brains of moribund and dead DDT-treated rainbow trout. This observation suggests that increased concentrations in the brain resulting from the relocation of DDT residues could have a deleterious effect on fish in natural aquatic communities.

Concentrations of DDT residues in eggs of lake trout and coho salmon from Lake Michigan were similar to those shown to adversely affect fish reproduction in natural waters (Burdick et al. 1964) and in the laboratory (Macek 1968). A mortality syndrome similar to that reported for lake trout fry hatched from eggs of DDT-contaminated lake trout from Lake George, New York (Burdick et al. 1964), has been observed in some coho salmon fry hatched from eggs of Lake Michigan fish (Johnson and Pecor 1969). Johnson and Pecor also noted that mortality during the first 8 wk was much higher among fry hatched from eggs of Lake Michigan origin (15–73%) than among fry hatched from eggs that came from Lake Superior or the west coast (about 1–5%). Pecor (1972) concluded that, although he could not show a positive correlation between DDT residue concentrations and mortality of different lots of coho salmon fry from Lake Michigan, his data did offer substantial evidence to support the relationship. He suggested that other toxic materials may also be involved in fry mortality. Despite occasional high mortality in hatcheries, survival of fry from eggs collected from Lake Michigan coho salmon has been considered adequate to provide yearling fish for restocking, and natural reproduction has occurred in some tributaries of lakes Michigan and Superior (Parsons 1973).

Although there is evidence that lake trout reproduce each year in Lake Superior, reproduction in Lake Michigan has not yet been documented (the first major spawning of stocked fish in Lake Michigan was in the fall of 1971); however, in both Wisconsin and Michigan, eggs stripped from Lake Michigan lake trout have been hatched and the fry have been successfully reared in hatcheries (Great Lakes Fishery Commission 1971).

It is difficult to study the effects of DDT residues on the reproduction of Great Lakes fish without also considering the effects of PCB's, which are present in relatively high concentrations in Lake Michigan fish. Work in Sweden suggested a relationship between the concentration of PCB's and egg mortality in salmon (Jensen et al. 1970). At present, however, there is no information concerning either the direct effects of PCB's on Great Lakes fish or the effects these materials may have through their interaction with DDT residues.

The average concentration of DDT residues in maturing coho salmon collected in Lake Michigan in the spring and fall of 1971 was lower than that in salmon collected during the previous 3 yr (Table 3). Similar decreases in 1971 were observed in lake trout, bloaters (*Coregonus hoyi*), and alewives collected from Lake Michigan near Saugatuck, Michigan (Reinert 1974). These declines in concentration have closely followed the general ban on the use of DDT that went into effect in 1970. Further sampling will be required, however, before it can be determined if these declines reflect a general lakewide trend, because of the many factors that must be considered in assessing concentrations of DDT residues in fish populations. The present study has shown that concentrations may vary widely with species, life stage, size, and tissue of fish, as well as with season and locality of collection.

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Composition and Nutritive Characteristics of Atlantic Canadian White Fish Meals

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REGIER, L. W., P. M. JANGAARD, H. E. POWER, B. E. MARCH, AND J. BIELY. 1974. Composition and nutritive characteristics of Atlantic Canadian white fish meals. J. Fish. Res. Board Can. 31: 201–204.

Three composite samples of whitefish meals, each representing samples over 4 mo from plants in eastern Canada were analyzed for proximate composition, minerals, vitamins, amino acids, available lysine, and pepsin digestibility. Protein quality was evaluated biologically in feeding trials with chicks. The composite samples were similar in chemical composition with respect to both macro- and micronutrients. In vitro pepsin digestibility was 95–96% and chemically estimated available lysine 6.5–6.9 g/16 g N, or 87–96% of the total lysine present. The growth response to the meals, when they were fed to supply either 4 or 8% of supplementary protein in diets containing a total of 14.5 or 18.5% of protein, respectively, indicated that the supplementary protein value was high.

REGIER, L. W., P. M. JANGAARD, H. E. POWER, B. E. MARCH, AND J. BIELY. 1974. Composition and nutritive characteristics of Atlantic Canadian white fish meals. J. Fish. Res. Board Can. 31: 201–204.

Les auteurs ont recueilli trois échantillons composites de farines de poisson à chair blanche dans des usines de la côte est du Canada. Ils en ont fait l'analyse par composants, dosé les minéraux, vitamines, aminoacides, lysine disponible et mesuré la digestibilité par la pepsine. Ils ont évalué biologiquement la qualité des protéines par des essais de nutrition avec des poulets. La composition chimique des échantillons est la même, tant sous le rapport des macro- que des microsubstances nutritives. La digestibilité par la pepsine est de 95–96%, et la lysine disponible, estimée chimiquement, est de 6.5 à 6.9 g/16 N, ou 87–96% de la lysine totale présente. La croissance en réponse à ces farines, données de façon à fournir 4 ou 8% de protéines supplémentaires dans un régime contenant un total de 14.5 ou 18.5% de protéines respectivement, démontre que la valeur des protéines supplémentaires de ces farines est élevée.

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THE need for detailed information about the nutrient composition of fish meals has become important because feed manufacturers are making extensive use of least cost formulation in designing rations for livestock and poultry. Accordingly, the Halifax Laboratory of the Fisheries and Marine Service has developed a program of sampling and evaluating commercial fish meals from defined raw materials produced on the Atlantic Coast of Canada. The results of a survey of herring meals have already been published (Power et al. 1969). The present paper presents the results of a similar study of white fish meals.

White fish meal by definition (Canada Department of Agriculture 1967) "is the clean, dried ground residue, containing not more than 4 percent of oil, from undecomposed, whole, non-fatty white-fleshed fish, including cod, haddock, hake, cusk, pollock, skates and monk fish, or cuttings thereof." Fish are usually gutted on board the trawlers at sea and the viscera (including liver) discarded. The fillets, representing approximately

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one-third of the weight of the fish are removed in the filleting plant. The residue, consisting of heads, backbone, and some cuttings, are processed to meal. Both presscake meal and whole meal (i.e. with evaporated presswater added back to the meal in the dryer) are made.

There are a number of reports regarding the chemical and nutritive characteristics of white fish meals manufactured in different countries. The composition and nutritional values of a commercial sample of Canadian Atlantic white fish meal, as compared to herring and freshwater fish meals, were studied by March et al. (1966). March et al. (1967) evaluated the effect of white fish meal on hatchability. The Association of Fish Meal Manufacturers in Britain has published a booklet containing data on white fish meals produced in that country and Arnesen (1969) carried out various analyses on Icelandic meals. Brueggemann et al. (1969) also evaluated samples of Icelandic meals produced from cod only. Barber et al. (1964) and Bunyan and Woodham (1964) carried out a wide range of nutritional studies on a sample of white fish meal and Pritchard and Wraige (1953) studied the contents of the vitamin B complex in British white fish meals. Miller (1970) has reviewed the literature and tabulated data on the available amino acid content of fish meals and a comprehensive bibliography on fish meal was recently published (Anon. 1970).

Experimental

Sampling

Monthly samples of white fish meal were collected from seven manufacturing plants in the Atlantic provinces of Canada over a period of a year. All the plants employed steam-drying systems. The samples, approximately 454 g (1 lb) each, were pooled into three composite samples, each of which represented 4 mo production. The meal samples were mixed for a minimum of 6 h in a rotating drum mixer equipped with baffles. Before mixing was begun, the mixer was flushed with nitrogen. Samples of the composite meals were stored in polyethylene bags.

Analytical Methods

Standard methods (AOAC 1965) were used for the determination of moisture, ash, ether-extract, nitrogen, thiamin, folacin, niacin, riboflavin, molybdenum, and iodine. For other nutrients the following methods were employed: vitamin B_{12} (USP 1965), choline (Horowitz and Beadle 1943), biotin (Wright and Skeggs 1944), pantothenic acid (Neilands and Strong 1948), amino acids (Moore et al. 1958), selenium (Gutenmann and Lisk 1961), and sulphur (Blanchar et al. 1965). The above analyses were carried out by the WARF Institute, Inc., Madison, Wisc. Available lysine was estimated by the chemical procedure of Carpenter (1960). In vitro protein digestibility was determined using pepsin (AOAC 1965).

Biological Tests

The general method followed for assessing supplementary protein values has been described previously (March et al. 1966). Some modifications were, however, made in the details of the procedure. Each meal was used to supply 4 or 8% of supplementary protein to wheat-based diets containing a total of 14.5 or 18.5% of protein. The reference protein was isolated soybean protein and DL-methionine was added to the reference protein diets at levels of 0.33 and 0.42%. Each test diet was fed to triplicate lots of 20 chicks from 3 to 31 days of age.

The procedure followed for determination of metabolizable energy value (March et al. 1965), employed 3-wk-old White Leghorn cockerel chicks. In the assay the white fish meals were substituted in the basal diet at a level of 25%.

Results and Discussion

The proximate composition of the three composite white fish meals was:

		Moisture	Ether-extract	Protein	Ash
1	air-dry	4.9	2.9	70.0	23.2
	dry wt		3.0	73.6	24.4
2	air-dry	5.0	3.2	68.8	23.7
	dry wt		3.4	72.4	24.9
3	air-dry	4.8	4.2	68.1	22.2
	dry wt		4.4	71.5	23.3
Avg	air-dry	4.9	3.4	68.9	23.0
	dry wt		3.6	72.5	24.2

The sample numbers represent meals collected during successive 4-mo periods beginning in January. The data indicate very little seasonal difference. Cod, haddock, hake, and pollock, which are the major raw materials for white fish meal, have a low oil content in the flesh with the liver being the major storage tissue for oil. According to Jangaard et al. (1967) seasonal variations in carcass reflecting reproductive state and availability of food are reflected principally in the size and oil content of the liver. As was noted previously, the livers are generally discarded at sea. The uniformity of the composition of the meals throughout the year is therefore consistent with these observations.

The results of the mineral analyses in Table 1 are consistent with the high bone content of the raw material from which white fish meals are

manufactured. Some differences in the proportions of the various mineral elements would be expected between meals made from different raw materials because of the differences in the mineral composition of the skeletal and soft tissues. Some differences might also be expected among meals depending upon the extent to which soluble mineral compounds are lost in the presswater if these solubles are not added back to the meal. As evidenced by the data of Table 1, white fish meal contains significant amounts of nutritionally essential trace elements.

TABLE 1. Mineral analyses of Canadian Atlantic white fish meals.

	1	2	3	Avg
		%		
Calcium	5.5	5.5	5.3	5.4
Magnesium	0.17	0.19	0.17	0.18
Phosphorus	2.6	2.7	2.5	2.6
Potassium	1.1	1.1	0.88	1.0
Sodium	1.2	1.3	0.83	1.1
Sulfur	0.59	0.49	0.41	0.50
		μg/g (ppm)		
Aluminum	123	123	123	123
Barium	2.5	<2.0	3.1	<2.5
Boron	13	13	12	13
Chromium	9.9	9.1	9.9	9.6
Cobalt	<0.05	<0.05	<0.05	–
Copper	6.4	6.4	6.4	6.4
Iodine	59	81	65	68
Iron	80	60	115	85
Manganese	11	11	7.4	9.8
Mercury	0.23	0.23	0.26	0.24
Molybdenum	<0.05	0.2	<0.05	–
Selenium	2.9	1.6	1.6	2.0
Strontium	>200	>200	>200	>200
Zinc	63	60	70	64

TABLE 2. Vitamin analyses of Canadian Atlantic white fish meals — μg/g (ppm).

Vitamin	1	2	3	Avg
Thiamine	2.6	2.2	1.5	2.1
Riboflavin	6.6	6.1	5.2	6.0
Pantothenic acid	9.90	11.6	8.38	9.96
Niacin	55.0	54.0	37.9	49.0
Choline chloride	4800	4850	5900	5180
Biotin	0.119	0.0932	0.157	0.123
Folacin	0.242	0.244	0.285	0.290
Vitamin B-12	0.0724	0.0734	0.097	0.081

The results of analyses of the meals for various vitamins of the B-complex are shown in Table 2. Levels of riboflavin, pantothenic acid, niacin, biotin, folacin, and vitamin B-12 were generally lower in the white fish meals than in meal made from whole herring (Power et al. 1969). The difference in the levels may be due to the presence of

TABLE 3. Amino acid analyses of Canadian Atlantic white fish meals — g/100 g protein.

	1	2	3	Avg
Lysine	7.50	6.86	7.50	7.29
Histidine	2.50	2.20	2.41	2.37
Arginine	6.50	6.80	6.80	6.71
Aspartic Acid	9.30	8.76	9.59	9.23
Threonine	4.15	3.88	4.30	4.12
Serine	5.17	4.42	4.68	4.76
Glutamic Acid	13.30	13.09	13.98	13.52
Proline	5.26	5.52	5.52	5.34
Glycine	10.45	10.69	9.56	10.63
Alanine	6.88	7.24	6.34	7.00
Valine	4.40	4.17	4.70	4.47
Methionine	2.80	2.70	3.02	2.85
Isoleucine	3.90	3.59	4.36	3.96
Leucine	6.88	6.50	7.43	7.00
Tyrosine	3.21	3.01	3.64	3.27
Phenylalanine	3.64	3.46	3.95	3.70

TABLE 4. Protein availability in Canadian Atlantic white fish meals.

Sample	Pepsin digestibility[a] (% *of crude protein*)	Available lysine (% *of crude protein*)
1	95.1	6.5
2	96.2	6.6
3	96.2	6.9
Avg	95.8	6.7

	Supplementary protein value	
Sample	S.P.V. 4%[b]	S.P.V. 8%[b]
1	116	106
2	111	105
3	118	106
Avg	115	106

[a]Using 0.2% of a 1:10,000 preparation.
[b]The samples were included in the diets to supply 4 or 8% of the protein.

viscera, including livers, in the meal made from whole fish.

The results of the amino acid analyses are given in Table 3, and the values for chemically determined available lysine in Table 4. Pepsin digestibility of the meal protein is also shown in Table 4. A lower proportion of muscle protein in the white fish meal in comparison with the amount present in whole fish and relatively larger amounts of collagen account for some differences between the amino acid composition of white fish meal and herring meal. Thus, the level of lysine was somewhat lower and that of glycine higher in white fish meals. Availability of the lysine was high and the pepsin digestibility of the total protein was 95–96%.

The supplementary protein values obtained from the chick assays (Table 4) indicate a favorable response to white fish meal relative to soybean protein meal when used to supplement wheat protein.

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The Helminth Parasites of Embiotocid Fishes. III. A New Species of the Genus *Cucullanellus* Törnquist, 1931 (Nematoda: Cucullanidae) from the Shiner Perch, *Cymatogaster aggregata* Gibbons

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WALDER, G. L., AND H. P. ARAI. 1974. The helminth parasites of embiotocid fishes. III. A new species of the genus *Cucullanellus* Törnquist, 1931 (Nematoda: Cucullanidae) from the shiner perch, *Cymatogaster aggregata* Gibbons. J. Fish. Res. Board Can. 31: 205–209.

Cucullanellus kanabus sp. nov., parasitic in the intestine of the embiotocid fish, *Cymatogaster aggregata* Gibbons from Departure Bay, British Columbia is described and illustrated. The main features distinguishing *C. kanabus* from previously described species in the genus are the arrangement of the caudal papillae in males, the general body shape and various measurements. The species considered referable to *Cucullanellus* Tornquist 1931 are listed.

WALDER, G. L., AND H. P. ARAI. 1974. The helminth parasites of embiotocid fishes. III. A new species of the genus *Cucullanellus* Törnquist, 1931 (Nematoda: Cucullanidae) from the shiner perch, *Cymatogaster aggregata* Gibbons. J. Fish. Res. Board Can. 31: 205–209.

Les auteurs donnent une description, avec illustrations, de *Cucullanellus kanabus* sp. nov., parasite de l'intestin de l'embiotocidé *Cymatogaster aggregata* Gibbons de la baie Departure, Colombie-Britannique. Les principaux caractères qui distinguent *C. kanabus* des espèces de ce genre décrites antérieurement sont l'agencement des papilles caudales chez les mâles et la forme générale du corps. Ils énumèrent les espèces qu'ils considèrent comme appartenant au genre *Cucullanellus* Törnquist, 1931.

Received July 24, 1973 Accepted October 31, 1973

A SPECIES of the family Cucullanidae from the shiner perch *Cymatogaster aggregata* Gibbons from Departure Bay, B.C. was reported by the second author (Arai 1969) and, following Campana-Rouget (1957), referred to the genus *Cucullanus* Müller, 1777. Further examination of the material has cindiated that it represents a previously undescribed species now referable to the genus *Cucullanellus* Törnquist, 1931. The following description is based on the same specimens.

Specimens were collected from fresh and frozen fish, heat- or cold-killed, and preserved in a mixture of glycerine-ethyl alcohol. The nematodes were either cleared in glycerine and examined in temporary mounts or stained with Delafield's haematoxylin. Stained specimens were either cleared in xylene and mounted in Canada balsam, or cleared in methyl salicylate and examined in temporary mounts. Serial transverse and frontal sections of the head region were also prepared.

All measurements in the following description are based on 32 males and 16 mature (egg-bearing) females unless otherwise stated. Only glycerine-cleared specimens were used for measurements. Unless otherwise indicated, all measurements are in millimeters. Figures were drawn with the aid of a drawing tube. Figures 1–3 were drawn from stained specimens cleared in methyl salicylate. All other figures were drawn from glycerine-cleared specimens.

Cucullanellus kanabus sp. nov. (Fig. 1-12)

MALES

Body 3.05–7.48 long; maximum width 0.084–0.20, near posterior end of esophagus. Head not enlarged, 0.098–0.15 wide. Posterior end curved strongly ventral, tail 0.065–0.11 long. Cuticle 0.002–

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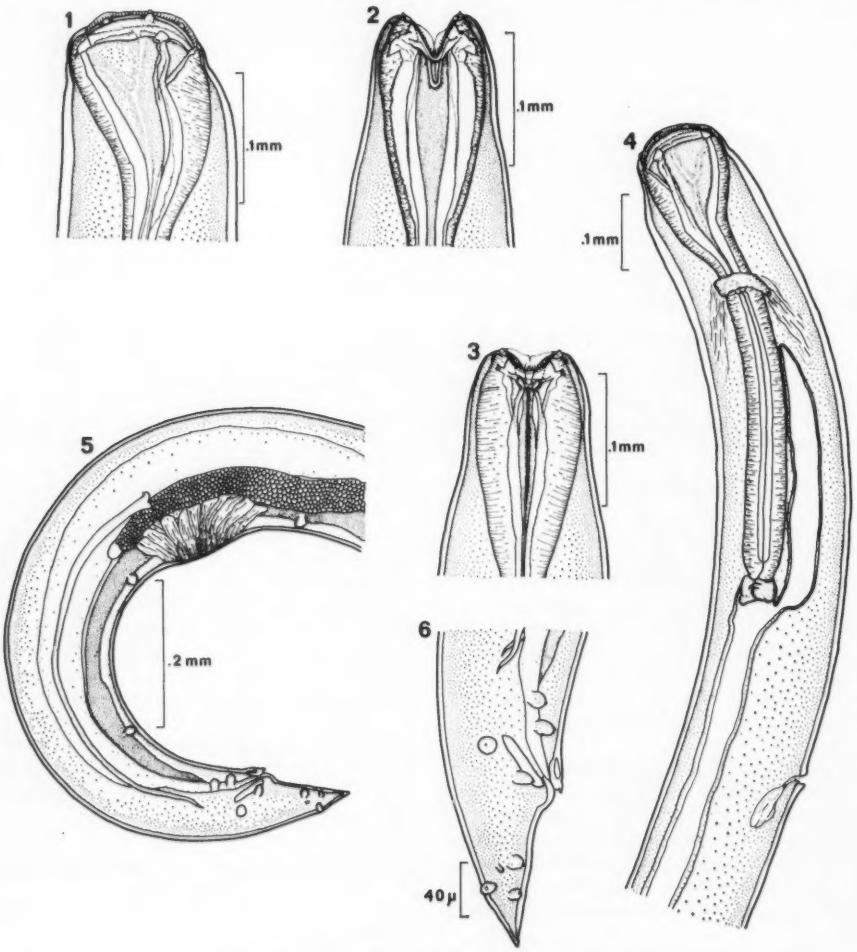
0.009 thick, somewhat thicker near anterior end. Fine striae 3–4 μm apart, visible only in larger specimens. Mouth a dorso-ventral slit, transverse to body axis, surrounded by a short collarette bearing many small teeth. Two lateral amphids and four submedian cephalic papillae present. Esophagus with sclerotized lining, dilated anteriorly as a laterally compressed pseudobuccal capsule. Dorsally, lining of capsule produced into lateral plates appearing in dorsal view as a pair of anterior horn-shaped structures and a pair of broad, blade-like structures tapering posteriorly and extending almost to level of nerve ring. Anterior portion of pseudobuccal capsule a thickened sclerotized rim containing a short angular block ventrally. Esophagus 0.47–0.71 long, practically not enlarged posteriorly, anterior diameter 0.090–0.14, midlength diameter 0.033–0.060, posterior diameter 0.037–0.068. Esophago-intestinal valve consisting of two lateral lobes. Anterior intestinal caecum ventral, 0.14–0.37 long. Nerve ring 0.14–0.27 from anterior end. Excretory pore 0.030–0.48 behind esophagus. Cervical papillae anterior or posterior to posterior end of esophagus, left papilla 0.24–0.79 and right papilla 0.35–0.80 from anterior end. Two lateral

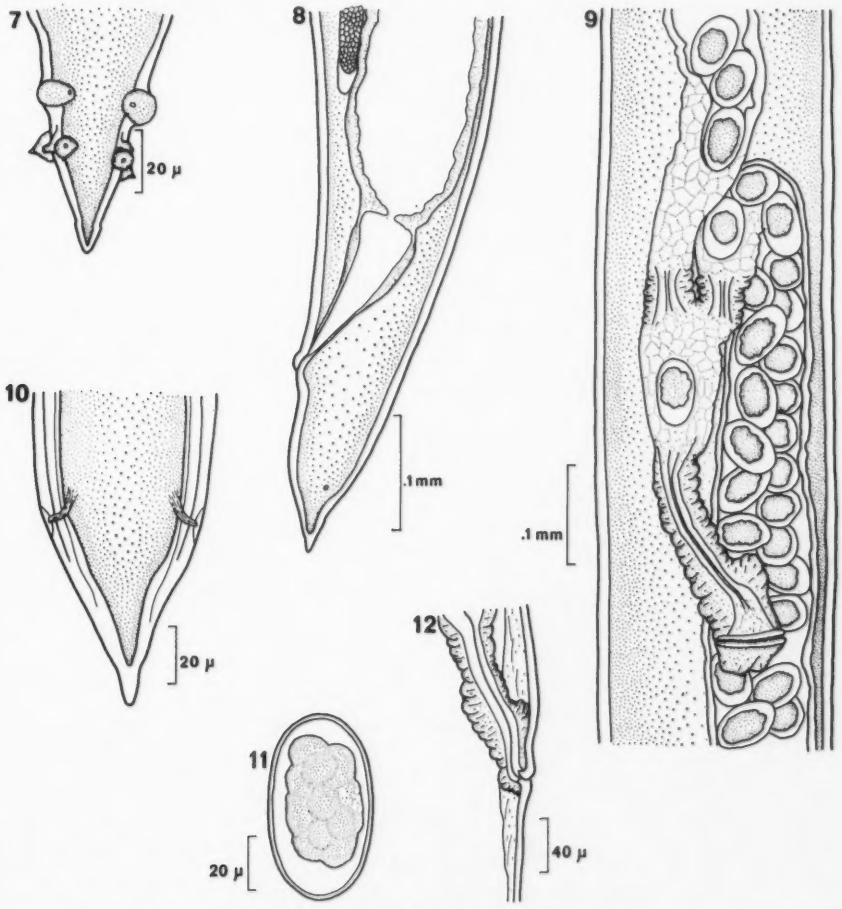


FIGURES 1–6. *Cucullanellus kanabus* sp. nov.
FIG. 1. Head of male, lateral view; FIG. 2. Head of male, dorsal view; FIG. 3. Head of male, ventral view; FIG. 4. Anterior end of male, lateral view; FIG. 5 and 6. Posterior end of male, lateral view.

papillae present in middle region of body; left papilla 1.07–2.00 and right papilla 1.96–3.87 from posterior end (in five specimens, 4.20–7.48 long). Preanal sucker 0.041–0.14 long (maximum extent of muscles), posterior margin 0.23–0.48 from anus (measured ventrally). Testis not convoluted, origin near level of, or posterior to, preanal sucker, anterior extent 0.14–2.25 from esophagus. Spicules slender, pointed; left spicule 0.33–0.60 and right spicule 0.32–0.61 long, extending to about middle of preanal sucker when completely retracted. Gubernaculum 0.034–0.059 long, 0.0059–0.010 wide. Eleven pairs of caudal papillae. Three pairs preanal, subventral: first pair just anterior to preanal sucker, second pair just posterior to sucker, third pair about $\frac{1}{3}$–$\frac{2}{3}$ distance from sucker to anus. Four pairs adanal: two pairs subventral just anterior to anus, third pair lateral, at level of or slightly posterior to second pair, fourth pair subventral, just posterior to anus. Four pairs postanal, near tip of tail: two pairs subventral, one pair subdorsal, at level of or slightly anterior to second subventral pair, one pair lateral, much smaller, between two subventral pairs.

Females

Body 4.99–10.61 long; maximum width 0.12–0.27, near posterior end of esophagus. Head not enlarged,



FIGURES 7–12. *Cucullanellus kanabus* sp. nov.
FIG. 7. Tail of male, ventral view; FIG. 8. Posterior end of female, lateral view; FIG. 9. Vagina and uteri, ventral view; FIG. 10. Tail of female, ventral view; FIG. 11. Egg; FIG. 12. Vulva, lateral view.

0.11–0.16 wide. Cuticle 0.003–0.009 thick, somewhat thicker near anterior end. Fine striae 3–4 μm apart, visible only in larger specimens. Mouth and pseudobuccal capsule structures as in males. Esophagus 0.57–0.77 long, enlarged posteriorly more than in males, anterior diameter 0.11–0.15, midlength diameter 0.046–0.071, posterior diameter 0.053–0.084. Intestinal caecum 0.17–0.42 long. Nerve ring 0.22–0.27 from anterior end. Excretory pore 0.13–0.46 behind posterior end of esophagus. Cervical papillae anterior or posterior to posterior end of esophagus, left papilla 0.50–0.92 and right papilla 0.48–0.85 from anterior end. Two lateral papillae present in middle region of body: left papilla posterior to vulva, 1.98–2.92 from posterior end; right papilla anterior to vulva, 3.38–5.15 from posterior end (in five specimens, 5.98–9.31 long). One pair of lateral phasmids, not papillose, present about ⅓ the distance from posterior end to anus. Vulva in posterior half of body, 3.26–6.11 from anterior end. Vagina directed anteriorly, uteri opposed. Eggs 59–74 μm long and 34–58 μm wide. Ovaries convoluted, anterior ovary extending to within 0.19–1.24 of esophagus, and posterior ovary to within 0.066–0.69 of anus.

Host — *Cymatogaster aggregata* Gibbons.

Habitat — intestine.

Locality — Departure Bay, B.C.

Specimens deposited — Holotype male (No. 216), allotype female (216a), and eight paratypes (216b–216i) deposited in the National Museums of Canada, Ottawa, Ont.

The name *kanabus* is from the Greek *kanabos*, meaning a lean or slender one.

Discussion

The genus *Cucullanellus* was erected by Törnquist (1931) to accommodate those species of Cucullanidae with a ventral intestinal caecum. Campana-Rouget (1957) regarded the preanal sucker and intestinal caecum present in some members of the family as being of no taxonomic importance at the generic level and suppressed several genera including *Cucullanellus*. However, Yamaguti (1961) and Maggenti (1971) both retained *Cucullanellus* as a valid genus. We are in agreement with this latter view. Accordingly, the new species is referred to *Cucullanellus* because of the presence of a preanal sucker, a ventral intestinal caecum, two ovaries in females, and 11 pairs of caudal papillae in males.

Törnquist (1931) and Yamaguti (1961) listed most of the species in the genus *Cucullanellus*. Two additional species, *C. amaruincai* and *C. rodriguesi*, have since been described by Teixeira de Freitas et al. (1969) and Pinto et al. (1970), respectively.

The genus *Cucullanellus* now appears to include the following: *C. abbreviatus* (Rudolphi, 1819) Törnquist, 1931; *C. adriaticus* Törnquist, 1931; *C. amaruincai* Teixeira de Freitas, Vicente and Ibañez, 1969; *C. anceps* (Gendre, 1927) Törnquist, 1931; *C. branchiostegi* Yamaguti, 1941; *C. cnidoglanis* Johnston and Mawson, 1945; *C. dichelyneformis* Szidat, 1950; *C. elongatus* Törnquist, 1931; *C. fraseri* (Baylis, 1929) Törnquist, 1931; *C kanabus* sp. nov.; *C. minutus* (Rudolphi, 1819) Törnquist, 1931; *C. nanus* Törnquist, 1931; *C. pleuronectidis* Yamaguti, 1935; *C. rodriguesi* Pinto, Fabio and Noronha, 1970; *C., sheardi* Johnston and Mawson 1944; *C. tripapillatus* (Gendre, 1927) Törnquist, 1931.

Cucullanellus kanabus most closely resembles *C. elongatus* and *C. tripapillatus*. It differs from *C. elongatus* in that the phasmids of the females are not papillose, the preanal sucker is much smaller, the spicules are shorter, and the males have four adanal pairs of papillae of which three pairs are subventral. *C. kanabus* differs from *C. tripapillatus* in that it is more slender, has a considerably shorter tail, has a slightly shorter esophagus, the nerve ring is more anterior, the cuticle is thinner and has shorter spicules, and the arrangement of the postanal papillae in the male is different. *Cucullanellus kanabus* differs from all known species of the genus except *C. rodriguesi* in being more slender. The length to width ratio of *C. kanabus* is 31–48 (mean 40.9) in females and 27–47 (mean 34.7) in males. The length to width ratio of *C. rodriguesi* appears to be about 45 in females and 40–42 in males. *C. kanabus* differs from *C. rodriguesi* in the number of caudal papillae in males and in the size and shape of the gubernaculum. Although the body widths of *C. cnidoglanis* and *C. sheardi* were not given in the descriptions of Johnston and Mawson (1944, 1945), it is evident from illustrations accompanying these descriptions that both species are relatively stout worms.

The sclerotized structures of the pseudobuccal capsule of *Cucullanellus kanabus* resemble closely those described by Berland (1970) in *Cucullanus cirratus* Müller 1777; *C. heterochrous* Rudolphi, 1802; and *Cucullanellus minutus*. One major difference, however, is that the "reniform structures" described by Berland are absent in *C. kanabus*. Campana-Rouget (1957) stated that the reniform structures of the capsule are poorly developed in *C. tripapillatus* and absent in *C. elongatus*. *C. minutus* has two pairs of these structures (Berland 1970). Although evidently of taxonomic value, unfortunately the pseudobuccal structures have been poorly described in most other works, so that comparisons with other species of *Cucullanellus* are difficult.

Acknowledgments

We express our appreciation to Dr L. Margolis of the Fisheries Research Board of Canada, Pacific Biological Station, Nanaimo, for laboratory and library facilities during part of this study and for access to his extensive personal files and literature. This work was supported in part by a National Research Council of Canada grant to the second author.

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NOTES

Evidence for the Lack of By-Pass Shunting in Teleost Gills[1]

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CAMERON, J. N. 1974. Evidence for the lack of by-pass shunting in teleost gills. J. Fish. Res. Board Can. 31: 211–213.

Retention of 15 μ diameter tracer-labelled microspheres in the gills was studied in some Alaskan teleosts. Neither adrenaline nor acetylcholine significantly affected the percentage retention, as predicted by earlier reports on by-pass shunts affected by these drugs. Rentention of 50 μ microspheres was nearly complete, even under conditions reported to favor shunting. Histological examination of microsphere-treated gills revealed no accumulation in the reported shunt pathways under any conditions. If the shunts do exist, their significance would seem to be slight.

CAMERON, J. N. 1974. Evidence for the lack of by-pass shunting in teleost gills. J. Fish. Res. Board Can. 31: 211–213.

Nous avons étudié, chez quelques téléostéens d'Alaska, la rétention dans les branchies de microsphères de 15 μ de diamètre marquées d'un traceur. Ni l'adrénaline ni l'acétylcholine n'affectent de façon significative le pourcentage de rétention, comme l'avaient déjà prédit des études sur les systèmes de dérivation affectés par ces drogues. La rétention des microsphères de 50 μ est presque totale, même dans des conditions que l'on sait propices au shuntage. L'examen histologique des branchies traitées aux microsphères ne révèle aucune accumulation, quelles que soient les conditions, dans les voies de shuntage signalées. S'il y a shuntage, il paraît plutôt insignifiant.

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SINCE the reports of Steen and Kruysse (1964) and the later confirmation by Richards and Fromm (1969), the existence of by-pass (nonrespiratory) shunts in teleost gills has been widely accepted in physiology literature. No one has yet considered the effect on arterial saturation of shunts by-passing the respiratory exchange surface; any large diversion of blood through nonrespiratory pathways would result in a significant decrease in arterial saturation. Due to the nature of the dissociation curve in rainbow trout, for example, a 20% admixture of venous blood (40% saturated) with the arterial stream would lead to a decrease in arterial P_{O_2} from about 95 to about 45 torr (Cameron 1971). So far, arterial tensions reported from teleosts under various normoxic conditions have been uniformly high, indicating at least 90% saturation (Randall 1970; Cameron et al. 1971).

In the course of my general studies on distribution of blood flow, I noticed some peculiarities in the distribution of microspheres in the gills and body. This led me to look more closely at the distribution of the microspheres in the gill filaments themselves, particularly to determine if any were being diverted through by-pass shunts.

Materials and methods — The experiments were performed with arctic grayling (*Thymallus arcticus*), burbot (*Lota lota*), and long-nosed suckers (*Catostomus catostomus*) obtained locally in the Chena River by electro-shocking. After a holding period in the laboratory, the fish were anesthetized lightly in (1:20,000) M.S. 222 (Sigma Biochemical Co.) and transferred to an operating table equipped with a pump for gill perfusion (Smith and Bell 1964). The pericardial cavity was then opened by blunt dissection, and PE 60 tubing introduced into the ventricle or ventral aorta for injection of drugs and microspheres.

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For most experiments, the procedure was as follows: a suspension of 15-μ diameter microspheres (density 1.3 g/cm^3) in Cortland saline was injected in a volume of 0.2–0.4 ml, followed by a saline rinse. After 5–10 min, either adrenaline (AD) or acetylcholine (ACh) in saline was injected through the same cannula, and a second dose of microspheres followed within 1 min. Between 2 and 3 min after the second injection, the fish were killed by injection of KCl into the ventricle or by clamping the ventral aorta. Two different gamma labels (Chromium^{51} and Strontium^{85}) were used to distinguish the two injections. Doses of adrenaline were 15–25 μg, acetylcholine 20–50 μg; these doses have previously been shown to cause marked changes in blood pressure (J. N. Cameron unpublished data; Stevens et al. 1972; Steen and Kruysse 1964). The theory and application of microsphere techniques for circulation research has been thoroughly reviewed elsewhere (Wagner et al. 1969).

For histological studies the above routine was abbreviated: only one injection was given and the fish killed 2–3 min afterward. When using the 50-μ diameter microspheres, dextran was added to the saline to retard settling, and 50 μg of ACh was injected just prior to the microspheres which should promote shunting, according to Steen and Kruysse (1964).

Activity of the whole carcass was assayed in a crystal scintillation gamma counter, and this whole body count was taken as 100% of dose rate. The whole gill arches were then removed for separate assay, and the activity found was expressed as a percentage of the total dose. The Cr^{51} and Sr^{85} have quite different radiation spectra, and could be assayed independently in the same sample.

Some pieces of gill were put into Bouin's fixative, sectioned at 5 μ, and stained with hematoxylin-eosin for examination under the light microscope.

Results — The results of the study of retention of 15-μ diameter microspheres in the gills are shown in Table 1. Retention appears to be slightly decreased both by AD and ACh, but with the large variability among individual fish, the differences were not statistically significant. The difference appeared less for burbot and sucker, where N was larger.

The second series of injections employing ACh and 50-μ diameter microspheres yielded the following results: the mean percent of total dose retained in the gills of five suckers was 93.0 ± 2.8% and for three grayling was 97.6 ± 4.2% (mean ± standard deviation). Since a small amount of activity is usually retained around the injection site, and some may have remained "upstream" of the gills, virtually none of the 50-μ spheres passed through the gills.

Histological examination of gills injected with 15-μ and 50-μ diameter microspheres showed that most of them were trapped in the afferent end of the secondary lamellae. In no case, in any of the many sections examined, did I find microspheres in the central compartment, which Steen and Kruysse reported to be the alternate pathway for blood flow. Figure 1 shows a typical oblique section through a grayling filament that had been injected with a large dose of 15-μ spheres. The central compartment can be seen in the lower portion of the photograph; it is filled with blood cells, but no microspheres. The secondary lamellae are evident in the upper portion as light areas running across the filament. Blood flow is from left to right in the lamellae.

TABLE 1. Summary of the effects of acetylcholine (ACh) and adrenaline (AD) injection of the retention of 15-μ diameter microspheres in the gill. Under "Treatment", "Cr, ACh, Sr" means that Cr^{51}-labelled spheres were injected, then after 5–10 min, ACh followed by Sr^{85}-labelled spheres within 1-min of each other (full explanation in text). Retention is calculated as the activity of each isotope expressed as a percent of the whole carcass activity for that isotope. None of the differences were statistically significant (*t*-test, paired data).

Species	N	Treatment	Percentage retention (±SD)	Change (%)
Burbot	7	Cr, ACh, Sr	51.4±6.5, 52.1±4.2	+.7
	8	Cr, AD, Sr	46.6±5.8, 43.2±4.3	−2.4
Long-nosed sucker	6	Cr only	66.8±2.2	
	4	Sr, ACh, Cr	62.5±5.5, 51.0±9.6	−11.5
Arctic grayling	3	Sr, AD, Cr	88.3±3.1, 67.5±4.6	−20.8
	3	Sr, ACh, Cr	64.0±4.3, 27.0±10.3	−37.4

Discussion — Many factors influence the distribution of microspheres, including their size, density, suspension medium, geometry of the vessels, and capillary beds, and the distribution of the blood stream into which they are injected (Wagner et al. 1969). With the experiments performed as they were, all these factors except the last should have been constant. Preliminary investigations showed no difference when two differently labelled doses of microspheres were injected with no intervening treatment. The 15-μ diameter microspheres have a roughly Gaussian distribution about the mean stated size, with a standard deviation of about 2.5 μ. Since fish erythrocytes are relatively large (13 × 9 μ for grayling, Lieb et al. 1953), some will undoubtedly pass through the secondary lamellae, accounting for the 20–40% passage indicated by Table 1.

For a shunt pathway to become operational, conditions must change to make it a lower resistance pathway. There must be either more or larger pathways opened to blood flow. In either case, a significantly lower percentage of the microspheres would have been trapped in the secondary lamellae. Since this did not occur (Table 1), either there is no such shunt, or the percentage blood flow through it did not change with the administration of adrenaline or acetylcholine.

That virtually all 50-μ diameter microspheres are retained in the secondary lamellae (and none,

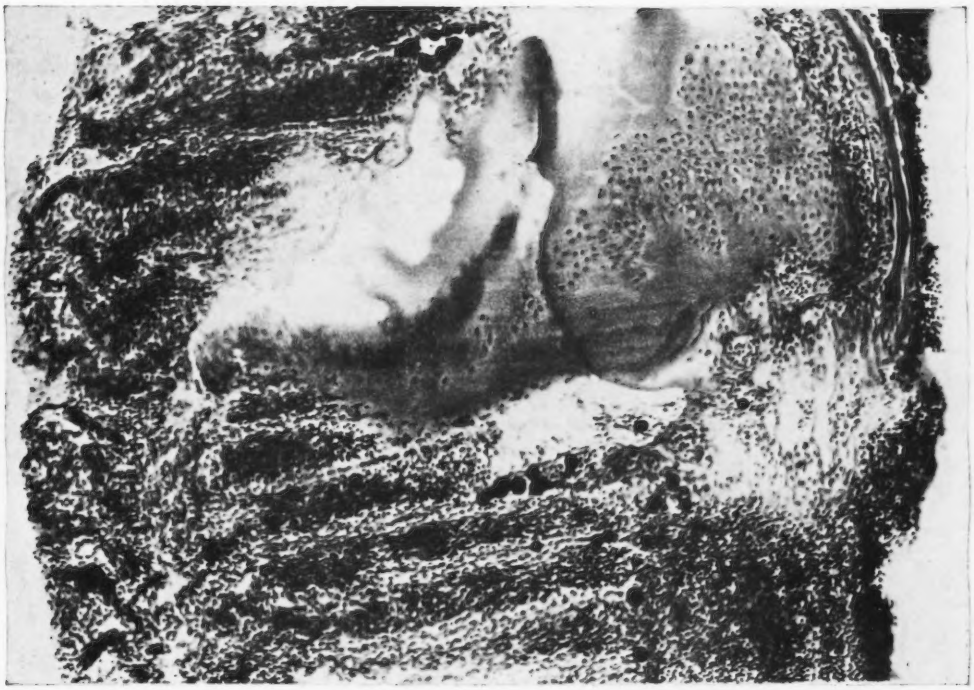

FIG. 1. Gill filament from a microsphere-injected Arctic grayling, sectioned parallel to the long axis of the filament in such a way that the secondary lamellae are transsected. The microspheres appear as opaque black circles lined up in the secondary lamellae. This fish had been injected with a very large dose of 15-μ diameter microspheres. Note the lack of microspheres in any other location, especially in the "central compartment" (translucent area in lower right).

Cameron — J. Fish. Res. Board Can.

evidently, in the central compartment) indicates that if there is a shunt pathway, there is virtually no blood flow through it, even after administration of acetylcholine, reported earlier (Steen and Kruysse 1964; Richards and Fromm 1969) to promote significant flow through the hypothetical shunt.

Finally, the histological evidence, though insufficient to prove a negative hypothesis, does not provide any evidence for a shunt, since microspheres appeared regularly and predictably in the afferent ends of the secondary lamellae, and nowhere else.

Work with 15-μ microspheres indicates no appreciable effect of AD or ACh on flow distribution of blood in the gill, studies with 50-μ spheres indicates nearly all flow is through small pathways, and histological examination failed to reveal any microspheres entering the "central compartment" described by Steen and Kruysse (1964). The presence of blood cells in this area lacks a clear explanation at present.

Perhaps a more reasonable theory for regulation of blood oxygenation in the fish gill is that the number of lamellae perfused varies (Davis 1972) and that ventilation-perfusion ratio is adjusted by this mechanism and by changing flow to the various gill arches (Cameron et al. 1971).

Acknowledgments—I express thanks to Mrs M. Mueller for able technical assistance, and to Mssrs D. Wilson and F. Mauer for collecting fish through the arctic winter.

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Comparison of Protection in Rainbow Trout (*Salmo gairdneri*) Inoculated with and Fed Hagerman Redmouth Bacterins

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ANDERSON, D. P., AND J. R. NELSON. 1974. Comparison of protection in rainbow trout (*Salmo gairdneri*) inoculated with and fed Hagerman redmouth bacterins. J. Fish. Res. Board Can. 31: 214–216.

Rainbow trout (*Salmo gairdneri*) fed 1.0 mg Hagerman redmouth bacterin per fish for 2 wk had no detectable specific, circulating, agglutinating antibody. In fish given a single subcutaneous inoculation of 1.0 mg of bacterin per fish, antibody was present from 3 wk later until 3 mo later, when the final sample was taken. Median lethal doses at various intervals after the bacterins were administered indicated that the inoculated fish could withstand a greater challenge by subcutaneous inoculation of the virulent bacteria than the orally immunized fish. The fish fed the vaccine lost their protection within 6 wk, whereas the inoculated fish had high levels of protection through 3 mo. The degree of protection was also confirmed by a "natural" exposure challenge.

ANDERSON, D. P., AND J. R. NELSON. 1974. Comparison of protection in rainbow trout (*Salmo gairdneri*) inoculated with and fed Hagerman redmouth bacterins. J. Fish. Res. Board Can. 31: 214–216.

Des truites arc-en-ciel (*Salmo gairdneri*) auxquelles on administre oralement 1.0 mg de la bactérine de la bouche rouge d'Hagerman pendant 2 semaines n'ont pas d'anticorps agglutinant spécifique décelable en circulation. Chez les poissons qui ont reçu une seule inoculation de 1.0 mg de bactérine, un anticorps apparaît 3 semaines après et persiste jusque dans le dernier échantillon, examiné après 3 mois. Les LD-50 observés à divers intervalles après traitement démontrent que les poissons peuvent résister à de plus fortes attaques lorsque la bactérie virulente est administrée par injection sous-cutanée plutôt que par la bouche. Les poissons qui ont reçu le vaccin par la bouche perdent leur immunité en dedans de 6 semaines, alors que les poissons inoculés possèdent encore un haut niveau de protection après 3 mois. Le degré de protection est également confirmé par exposition "naturelle" aux attaques de la bactérie.

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IMMUNIZATION against Hagerman redmouth disease (HRM) in rainbow trout (*Salmo gairdneri*) has been accomplished in previous laboratory experiments (Ross and Klontz 1965; Anderson and Ross 1972). Although trout fed vaccine have not been found to produce circulating, agglutinating antibody, protection in these fish was demonstrated by their ability to withstand challenge when high levels of bacteria were inoculated subcutaneously. Fish inoculated with the HRM vaccine produced circulating antibody levels which follow a well-established pattern for trouts (Krantz et al. 1964a; Anderson and Klontz 1970).

Very little background data exists comparing the oral and the inoculation routes of vaccination with fish. Krantz et al. (1964b) concluded that feeding bacterins to prevent furunculosis in a hatchery was unsatisfactory, but stated that the inoculation route might prove valuable for the prevention of furunculosis in special cases. Fujihara and Nakatani (1971) in immunization experiments with *Chondrococcus columnaris* worked with oral and intraperitoneal methods of vaccination but made no direct comparisons using the same species and age-groups of fish.

The following series of experiments were performed to compare the levels and durations of protection against redmouth in rainbow trout given equal amounts of bacterin orally and parenterally.

Materials and methods — The HRM bacteria for the vaccine preparation were taken from a bacterial stock stored on semisolid culture media in the laboratory. The original isolate was taken from a moribund rainbow trout in the Hagerman Valley region, Idaho in 1970. This isolate has been used in the preparation of previously successful HRM vaccines in this laboratory. The vaccine was prepared by growing the

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bacteria in a Virtis Model 43–100 fermenter containing 12 liters of trypticase soy broth. After 24 h of incubation, the cells were harvested using a continuous centrifuge, washed in saline three times and treated for 4 days with 3.0% chloroform as previously described by Anderson and Ross (1972).

Four hundred fifty fingerling rainbow trout weighing approximately 2 g apiece were brought to the laboratory from the National Fish Hatchery at Quilcene, Wash. The fish were divided into three equal groups and held in dechlorinated Seattle city water at temperatures ranging from 17–18 C. The first group was inoculated with the vaccine, a second was fed the vaccine, and the third was held as the unvaccinated controls.

The fish were fed a blank complete test diet (for formulation, see Halver 1957) for 1 wk prior to the time of inoculation or the beginning of the feeding of the vaccine.

The inoculated fish were given a single subcutaneous injection of bacterin immediately craniad to the dorsal fin origin with 0.1 ml of a suspension containing 1.0 mg of lyophilized cells. The fish fed the vaccine were given this same amount incorporated into the complete test diet over a 2-wk period.

Circulating antibody was measured in the fish at weekly intervals up to 5 wk after the vaccination, then monthly thereafter for 3 mo. At each sampling, five fish were taken from each group, caudal peduncles were severed, and the blood was collected in capillary tubes. Antibody was measured by the agglutinin technique in microtiter plates. The antigen for these agglutinin tests was the washed bacterin. In a few cases, where the collection of blood was minimal, the serums of some individuals were pooled.

Protection in the trout against the HRM pathogen was tested for using LD-50's calculated by probit analysis. Fish were challenged with virulent bacteria weekly for the first 5 wk and approximately monthly thereafter. The challenge method, described by Anderson and Ross (1972), consisted of inoculating 0.1 ml of 10-fold dilutions from an original suspension containing 4×10^8 bacteria per 0.1 ml into individual lots of five fish each. In addition, a "natural" exposure challenge was conducted 5 wk after the injection date. For this test five control fish were inoculated with a heavy suspension of the pathogen and placed into a tank with 10 fish from each of the original three groups. For the following 2 wk the course of the mortalities from the disease was recorded. Kidneys of all dead fish were cultured for the presence of the HRM pathogen to confirm the cause of death.

Results and discussion—Specific antibody was found in the inoculated trout 3 wk after the injection date (Fig. 1). It is interesting that these small fish were capable of this buildup of antibody and of sustaining detectable levels in their humoral systems for the entire length of this experiment. The mean antibody titer decreased by the end of the 3-mo experiment.

The fish fed the HRM vaccine did not develop detectable specific humoral antibody. Anderson and Ross (1972) and Fyrer et al. (1972) also have not been able to detect antibody in fishes given oral vaccines; yet in both previous studies, the fish

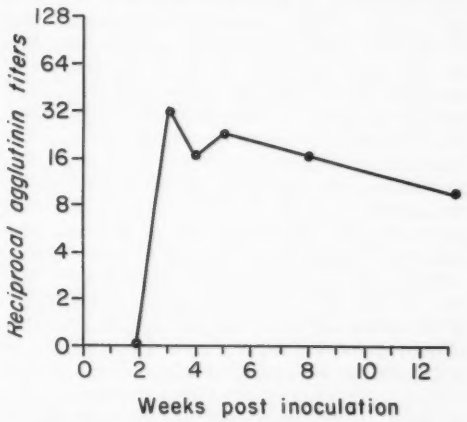

FIG. 1. Mean agglutinating antibody titers at various intervals for five trout inoculated with the HRM vaccine. The titers were measured by the microtiter method using chloroform-killed bacterial cells as the antigen. No specific antibody was found in the fish fed the vaccine.

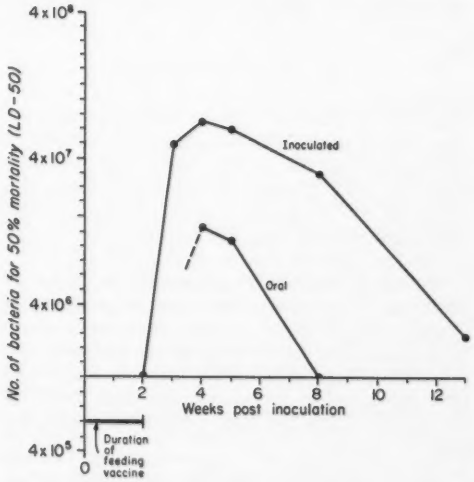

FIG. 2. Following vaccination, a rise in protection (LD-50's), shown by the resistance to increased doses of bacteria, was observed in the fed and inoculated groups of fish. The fish consistently had LD-50's of less than 4×10^5 bacteria per 0.1 ml.

demonstrated high levels of protection against the respective pathogens. The reasons for the absence of antibody in the orally vaccinated fish have not been established. One explanation may be in the amount of antigen made available to the fish's immune system. In the present series of experiments the 1.0 mg of antigen administered to each fish can be assumed to be a reasonably accurate total dose for the inoculated fish; however, the amount of antigen given to each fish by the oral route could not be regulated. In addition, nothing is known about the amount of antigen available after ingestion that actually reached appropriate areas of the fish to stimulate the protective immune response. In studies with hamsters, Dolezel et al. (1971) found no measurable serum antibody from orally administered antigens, but lymphoid cells reacted with the antigens in vitro, demonstrating the aquisition of a specific immune response. Chiller et al. (1969) inoculated sheep red blood cells into large rainbow trout and showed by a modified rosette technique that the lymphoid cells of these fish are active in an immune response against these antigens. In these studies they also detected circulating antibody at about the same time as the peak of the cellular response. Orally administered antigens may involve some similar cellular response in trout without production of large amounts of antibody or may stimulate localized antibody production primarily in the gastrointestinal tract. More sensitive techniques may possibly demonstrate low levels of circulating antibody in the orally immunized fish.

Protection against the HRM pathogen was demonstrated in both vaccinated groups of trout regardless of how the antigens were administered. The calculation of LD-50's indicated that the trout inoculated with the vaccine had higher levels of protection and retained it longer than those fish given the vaccine orally (Fig. 2). All control fish died when injected with the dilutions used to calculate the LD-50's for the vaccinated fish.

The LD-50 results were confirmed by the "natural" challenge. Within 2 wk after being exposed to fish which were moribund or dead as a result of an HRM infection, the 10 control fish had died. By the same challenge, none of the fish which had been vaccinated by inoculation had died, and 5 of the 10 fish which had been vaccinated orally had died.

Because the fish fed the vaccine were started on the 2-wk program the same day the other group of fish were inoculated, differences in the actual time of appearance of protection are difficult to compare. However, the orally vaccinated fish at the 8- and 13-wk sample dates had no more protection against the HRM challenge than the controls.

These experiments confirm that the inoculation route for the HRM vaccine is necessary under at least certain conditions for the stimulation of detectable circulating antibody. The inoculated fish not only showed higher levels of protection than the orally vaccinated fish but retained their levels of protection longer.

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Fecundity and Egg Size of a Brood Stock of *Salvelinus fontinalis* × *S. namaycush* Hybrids (Splake)[1]

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AYLES, G. B. 1974. Fecundity and egg size of a brook stock of *Salvelinus fontinalis* × *S. namaycush* hybrids (splake). J. Fish. Res. Board Can. 31: 217–220.

Estimates of average egg diameter and average number of eggs per female from a brood stock of *Salvelinus fontinalis* × *S. namaycush* (splake) hybrids were 0.468 cm and 1169 eggs, respectively. Variation in egg size between females was attributable to variation in both size and age of the fish, whereas differences in fecundity were attributed only to differences in female size. At a given size a splake had more and larger eggs than have been reported for lake trout. The significance of the findings is discussed in relation to the reestablishment of a viable trout population in Lake Huron.

AYLES, G. B. 1974. Fecundity and egg size of a brood stock of *Salvelinus fontinalis* × *S. namaycush* hybrids (splake). J. Fish. Res. Board Can. 31: 217–220.

Le diamètre moyen de l'oeuf et le nombre moyen d'oeufs par femelle dans un stock reproducteur d'hybrides *Salvelinus fontinalis* × *S. namaycush* (truite moulac) sont de 0.468 cm et de 1169 oeufs respectivement. Les variations de la grosseur de l'oeuf, d'une femelle à l'autre, sont fonction de la taille et de l'âge du poisson, alors que les différences de fécondité ne sont attribuables qu'aux différences de taille seulement. Pour une taille donnée, les oeufs de la truite moulac dépassent, en nombre et en grosseur, ceux du touladi. La discussion porte sur la signification de ces observations en rapport avec le rétablissement d'une population viable de truites dans le lac Huron.

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THIS report is an analysis of the fecundity of the brood stock of *Salvelinus fontinalis* × *S. namaycush* hybrids (splake) maintained at the selective breeding research facility of the Ontario Ministry of Natural Resources at Maple, Ont. The splake is being used for the rehabilitation of the trout fishery in Lake Huron (Budd 1957; Berst and Spangler 1970) and has been successfully introduced into several lakes (Martin and Baldwin 1960), but its fecundity has not been described.

Experimental procedures — The fish used (total 106) were fourth to sixth generation hybrids taken from the 1970 brood stock. The age of individual females was known (mean 2.55) and weight (mean 561 g) and fork length (mean 32.4 cm) were measured immediately prior to spawning.

Eggs were stripped from females and fertilized. After water hardening the total volume of eggs was measured (± 5 cm^3) in a graduated cylinder and 15–20 eggs were removed and preserved in Davidson's solution (3 parts 35% ethanol, 2 parts formalin, 1 part glacial acetic acid, 1 part distilled water). The average egg volume (mean 0.054 cm^3) was determined by measuring the water displaced in a 10-ml graduated cylinder by the addition of the 15–20 eggs, and the total number of eggs (mean 1169) was determined by dividing the total volume of eggs by the average egg volume.

A series of regression analyses were then performed on the data to determine the best model relating egg size and number of eggs to the independent variables weight, fork length, and age. A test of the quadratic of the independent variable was used to confirm that all simple regressions presented here are linear. There was a curvilinear relation between age and egg volume and a linear relation between $\log_{10}$ age and egg volume so the latter relation was used for the regression and partial regression analyses.

[1]Taken in part from a thesis submitted to the Zoology Department of the School of Graduate Studies, University of Toronto, November, 1972.

[2]Present address.

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Partial regression analyses were carried out to determine the effects of individual variables, independent of the other two variables, on the dependent variables.

Results — Approximately 58% of the total variation in egg size was attributable to variations in weight, fork length, or age of the female. The partial regression equations showing the relations between egg volume (EV, in cc) and female weight (W, in g), fork length (FL, in cm), and age (A, in years), and the correlation coefficients, were:

Equation	r
$EV = .0438 + .0000193\ W$	.756
$EV = .0183 + .00112\ FL$	.758
$EV = .0330 + .0506 \log_{10} A$	.750
$EV = .0380 + .0000109\ W + .0245 \log_{10} A$	.773
$EV = .0298 + .0000093\ W + .000602\ FL$	.765
$EV = .0235 + .000666\ FL + .0223 \log_{10} A$	.768
$EV = .0336 + .00000842\ W + .000230\ FL + .0208 \log_{10} A$	.774

The analyses indicated there was an effect of the size of the female, either weight or length, on the size of the egg independent of the age of the female. Within an age-group, larger fish had larger eggs; and within a size class, older fish had larger eggs.

To facilitate comparisons of egg size between splake, lake trout, and brook trout (Fig. 1) the following regression was calculated:

$$\text{egg diameter (cm)} = .0956 + .2489 \log \text{fork length (cm)}$$

Weight and length were significantly better indicators of fecundity (80–85% of the total variation was attributable to differences in size of the female) than was age (70%).

The partial regression equations showing the relations between number of eggs produced (EN) and female weight (W, in g), fork length (FL, in cm), and age (A, in years) were:

Equation	r
$\log_{10} EN = .308 + .995 \log_{10} W$	.929
$\log_{10} EN = 1.883 + 3.187 \log_{10} FL$	.910
$\log_{10} EN = 1.540 + 2.348 \log_{10} A$	.860

These analyses indicated there was no effect of age on fecundity, independent of the size of the female.

Discussion — My results show that in splake there are independent effects of both age and size of the female on the size of her eggs. Wydoski and Cooper (1966) found no effect of age, independent of length, on egg size in a population of brook trout (*Salvelinus fontinalis*) and Scott (1962) found no effect of length, independent of age of the fish, in rainbow trout (*Salmo gairdneri*). Possibly the present results are indicative of a hybrid population and are not typical of all salmonid populations.

Egg size is partially genetically determined in Atlantic salmon (*Salmo salar*) (Belding et al. 1932) and in some rainbow trout (Scott 1962). Data from other sources are limited and it is impossible to distinguish between environmental and genetic effects; but for a given size, brook trout have approximately the same size eggs as splake whereas lake trout (*Salvelinus namaycush*) have smaller eggs (Fig. 1). A large proportion of the differences in egg size between brook trout, splake, and lake trout may be the result of a difference in the size of the females.

At a given fork length the splake of this stock and the F_1 splake examined by Berst and Spangler (1970) and Martin and Baldwin (1960) are more fecund than lake trout populations but slightly less fecund than brook trout populations reported in the literature (Fig. 2). Brook trout mature at age 2–3 (Allen 1956) but lake trout females mature at age 5–8 (Alm 1959). After several generations of selection for early maturation in the hatchery, these splake ripen during their 2nd or 3rd yr (Ayles and Berst 1973) and may spawn when less than 25 cm in length.

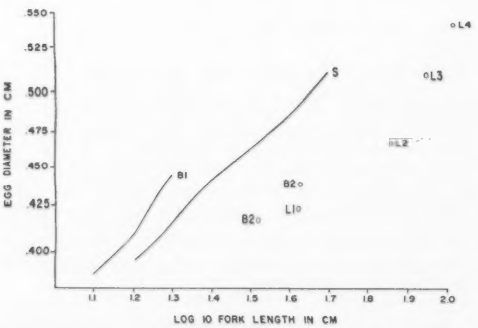

FIG. 1. The relation between fork length and the diameter of ripe eggs for lake trout, brook trout, and splake. Curves B1 and S are plotted from a regression equation, and mean egg sizes are given for other populations: B 1, brook trout (Wydoski and Cooper 1966); B 2, brook trout (Buss and McCreary 1960); L 1, lake trout (Lake Louisa, personal communication N. V. Martin, Ont. Ministry of Nat. Resources); L 2, lake trout (Lake Opeongo, personal communication N. V. Martin); L 3 and 4, lake trout (Eschmeyer 1955); S, splake (this report).

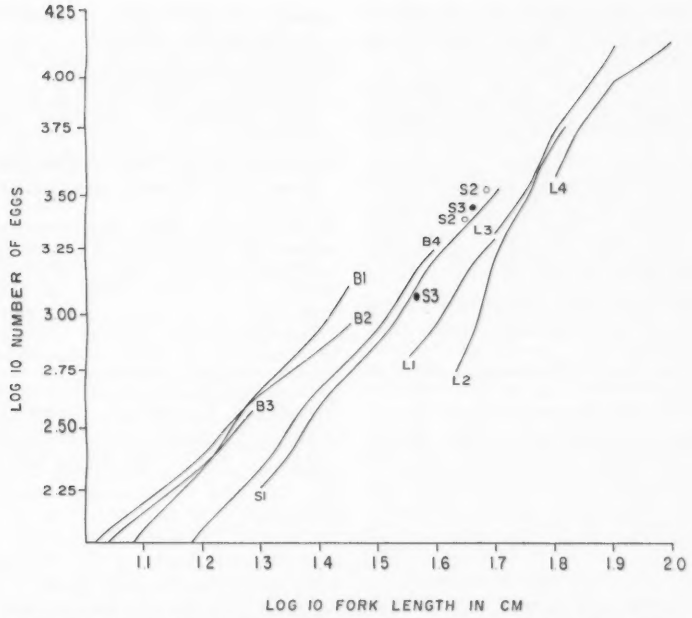

FIG. 2. The relation between fork length and the number of eggs produced by lake trout, brook trout, and splake. S2 and S3 are mean values, and other curves are replotted from the originals. B 1, brook trout (McFadden 1961); B 2, brook trout (Copper 1953); B 3, brook trout (Allen 1956); B 4, brook trout (Vladykov 1956); L 1, lake trout (Rahrer 1965); L 2, lake trout (Hanson and Wickwire 1967); L 3, lake trout (Lake Opeongo personal communication N. V. Martin); L 4, lake trout (Eschmeyer 1955); S 1, splake (this report;) S 2, splake (Berst and Spangler 1970); S 3, splake (Martin and Baldwin 1960).

In terms of fecundity, splake are better suited than are lake trout for use in the reestablishment of a viable reproducing trout population in Lake Huron. In South Bay, Lake Huron, lake trout did not mature before age 7 (Budd and Fry 1960), when they were about 58 cm long (Fry 1953). In Lake Huron, trout longer than 40 cm are vulnerable to both commercial fishing (Berst and Spangler 1970) and lamprey (*Petromyzon marinus*) predation (Budd and Fry 1960), and it has not been possible to reestablish a self-reproducing population of lake trout in Lake Huron (Budd and Fry 1960). Splake would mature and spawn once and possibly twice before being vulnerable to fishing or lamprey predation and at a given size would produce more eggs.

Acknowledgments — I wish to acknowledge the assistance of Mr J. Dewar and Mr D. Hull of the Ontario Ministry of Natural Resources, who provided much of the data and Mr P. Banks, who assisted in the analyses. Dr L. Butler of the University of Toronto and Mr A. H. Berst of the Ontario Ministry of Natural Resources kindly reviewed the manuscript. This investigation was supported, in part, by a National Research Council grant to Dr Butler.

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First Record of the Mooneye (*Hiodon tergisus*) in Alberta

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ROBERTS, W. E. 1974. First record of the mooneye (*Hiodon tergisus*) in Alberta. J. Fish. Res. Board Can. 31: 220–221.

Fourteen specimens of the mooneye (*Hiodon tergisus*) were collected from the North Saskatchewan and Red Deer rivers in central Alberta, and are first records of this species from these rivers, representing a considerable westward extension of its known range. There appears to be an established population in the Red Deer River, where 13 of 35 *Hiodon* specimens examined were *H. tergisus*, the remainder being goldeye (*H. alosoides*). Only one of fifty-five *Hiodon* specimens examined from the North Saskatchewan River was *H. tergisus*.

ROBERTS, W. E. 1974. First record of the mooneye (*Hiodon tergisus*) in Alberta. J. Fish. Res. Board Can. 31: 220–221.

Quatorze laquaiches argentées (*Hiodon tergisus*) ont été capturées dans les rivières Saskatchewan-Nord et Red Deer, en Alberta centrale. C'est la première mention de l'espèce dans ces rivières, une extension considérable vers l'ouest de son aire connue. La laquaiche argentée était connue dans la rivière Saskatchewan-Sud, mais non au nord ni à l'ouest de cette rivière. Il semble y avoir une population d'établie dans la rivière Red Deer, où 13 des 35 spécimens examinés étaient *H. tergisus*, le reste étant la laquaiche aux yeux d'or (*Hiodon alosoides*). Un seul des 55 *Hiodon* examinés dans la Saskatchewan-Nord était *H. tergisus*.

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FOURTEEN specimens of the mooneye, *Hiodon tergisus*, were collected in the Red Deer and North Saskatchewan rivers in Alberta during 1973. The first and second specimens were taken by the author while angling with a dry fly at the confluence of the Medicine and Red Deer rivers (52°04′N, 113° 28′W) on June 15, and from the North Saskatchewan River near Fort Edmonton Park, Edmonton, Alta. (53°33′N, 113°28′W), on July 10. Ten specimens were gillnetted at the Medicine River site in July. Three specimens were collected from the confluence of the Blindman and Red Deer rivers in July and August.

Paetz and Nelson (1970) did not report this species from Alberta but suggested it might be present. *Hiodon tergisus* had been known from the South Saskatchewan River in Saskatchewan (F. M. Atton, Saskatchewan Fisheries Laboratory, Saskatoon, personal communication) but not from the north and west of this. The mouth of the Medicine River is approximately 200 air miles (320 km) from the confluence of the Red Deer and South Saskatchewan rivers, and Edmonton is approximately 335 air miles (536 km) from the confluence of the North and South Saskatchewan rivers. The discovery of these specimens represents a considerable extension of their known range.

The first specimen was taken from turbid water (Secchi disc reading approximately 30 cm) with a slight current, at the confluence of the then turbid side channel of the Red Deer River with the tea-colored water of the Medicine River. Later collections at this site were from tea-colored water with a barely perceptible current, the side channel having ceased to flow. The North Saskatchewan specimen was taken from a quiet eddy with a Secchi disc reading of 0.5 m.

Meristic and morphometric data were obtained following the methods of Hubbs and Lagler (1964). All counts were taken from the left side of the fish, with the exception of those for gill rakers where the first right arch was employed. FL (range) 235–283 mm; wt (range) 139.0–295.2 g; D 12; A 27–31 (mode 29); lateral line scales 56–60 (mode 57); scale rows above lateral line 6 or 7 (mode 7); scales around caudal peduncle 16; gill rakers: upper arch 7–10 (mode 9), lower arch 9–11 (mode 10), total 17–20 (mode 19).

Hiodon tergisus might occur in the South Saskatchewan River system, the Red Deer River up to at least the mouth of the Medicine River, and in the North Saskatchewan River up to at least Edmonton. It has likely been collected in the past but overlooked due to its close resemblance to *H. alosoides*. The North Saskatchewan specimen may be an indication of recent extension of the range of this species in this drainage while the Red Deer River population has apparently been established for some time, specimens having been taken as long as 20 yr ago by local anglers (G. Pigeon, Innisfail, Alta. personal communication).

All mooneye specimens are deposited in the University of Alberta, Museum of Zoology (UAMZ #3153, 3154, 3163–3171, 3199).

Acknowledgments — Dr J. S. Nelson of the University of Alberta verified the author's identification of the specimens. Mr M. Kraft, Red Deer regional fisheries biologist, provided the Blindman River specimens.

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An Acoustic Estimate of the Escapement of Sockeye Salmon (*Oncorhynchus nerka*) into Lake Washington in 1971[1]

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THORNE, R. E., AND J. J. DAWSON. 1974. An acoustic estimate of the escapement of sockeye salmon (*Oncorhynchus nerka*) into Lake Washington in 1971. J. Fish. Res. Board Can. 31: 222–225.

The feasibility of estimating the escapement of sockeye salmon (*Oncorhynchus nerka*) into Lake Washington by hydroacoustics was explored during 1971. Surveys were made of large fish targets within the lake just before and after the spawning migration of sockeye salmon up the Cedar River. A decrease was observed after the spawning migration comparable to the estimated escapement as determined by weir counts and spawning ground surveys.

THORNE, R. E., AND J. J. DAWSON. 1974. An acoustic estimate of the escapement of sockeye salmon (*Oncorhynchus nerka*) into Lake Washington in 1971. J. Fish. Res. Board Can. 31: 222–225.

Nous avons exploré la possibilité d'estimer, par des méthodes hydroacoustiques, l'échappement des saumons nerka (*Oncorhynchus nerka*) dans le lac Washington en 1971. Nous avons dénombré les grands poissons cibles dans le lac immédiatement avant et après la montaison des saumons nerka dans la rivière Cedar. Nous avons observé, après la migration de fraie, un déclin comparable à l'échappement estimé par comptages dans les pêcheries et par recensements sur les frayères.

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THE run of sockeye salmon (*Oncorhynchus nerka*) into Lake Washington, developed from Baker Lake stock during 1935–45, increased considerably during the last decade and made possible a limited gillnet fishery in 1968 and 1969, and seine and recreational fisheries in 1971. A need arose for an accurate and rapid indication of run size for effective regulation of the harvest; therefore, the Washington State Department of Fisheries (WDF) began monitoring the size of the run by tagging and recapture, weir counts, and spawning ground surveys. Weir counts and spawning ground surveys are too slow for measuring escapement and regulating the catch. Tagging-and-recapture operations require considerable effort and yield imprecise estimates because of mortality (S. B. Mathews, Washington State Department of Fisheries, personal communication).

Since 1969, the Fisheries Research Institute in cooperation with WDF has been making acoustic surveys of abundance of juvenile sockeye salmon in Lake Washington. These surveys have shown extremely large echo targets in midwater during the summer months, correlated with the movement of adult sockeye salmon into the lake. Further, adult sockeye salmon have been captured frequently in a 3-m Isaacs-Kidd midwater trawl at depths of maximum observance of the large targets (Thorne and Woodey 1970). The feasibility of modifying the acoustic techniques to measure adult abundance was explored. The movement of adults into the lake is completed by late August and spawning migration up the Cedar River, the primary spawning area, does not begin until mid-September. The fish remain high in quality in the interim and are available to the fisheries within the lake as late as early September. An accurate estimate of abundance at this stage would provide a basis for the harvest of fish, except for those needed for spawning.

Materials and methods — Hydroacoustic data acquisition system — The basic components of this system are a Ross 200A Fineline echo sounder with a frequency of 105 kHz, a pulse length of 0.6 M/S, and a transducer producing a circular beam pattern of approximately 8° full angle at 6 dB down. The echo sounding data are converted from 105 kHz to 5 kHz by chopper and filter circuits and recorded by a standard stereophonic tape recorder. The system is described in detail in Thorne et al. (1972).

[1]Contribution No. 391, College of Fisheries, University of Washington, Seattle, Wash.

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Survey procedure — An initial series of transects was conducted on September 12, 1971, after recruitment of adult sockeye salmon into the lake ended and before migration into the Cedar River began. These transects were made after dark because sockeye salmon were known to migrate to, and disperse in, midwater at dusk. Effort was concentrated in the area south of Bailey Peninsula near the Cedar River (Fig. 1A), where most fish were expected to occur. Total sampling time at a speed of 7 knots (12.9 km/h) was about 3 h.

A second survey was conducted the night of November 10, after most sockeye salmon had moved up the Cedar River. The series consisted of 19 transects (Fig. 1B). A final survey, on December 7 after all adult sockeye left the lake, consisted of 18 transects (Fig. 1B). During all three surveys the transects were terminated at about the 18-m contour.

Data analysis — To separate the echoes of adult sockeye we established a threshold just above the largest of the small echoes associated with juvenile sockeye salmon and other small fish (Fig. 2) and counted the individual fish echoes exceeding the threshold in each transect. Threshold level decreased in proportion to depth. The threshold was used with the 20 log R Time-Varied-Gain of the sounder to correct for the effect of depth on the echo strength of the fish targets (Thorne 1972).

The sampling area of the sounder was estimated by modification of the trace length method (Forbes and Nakken 1972). The average of successive echoes from individual large fish targets was determined as the vessel

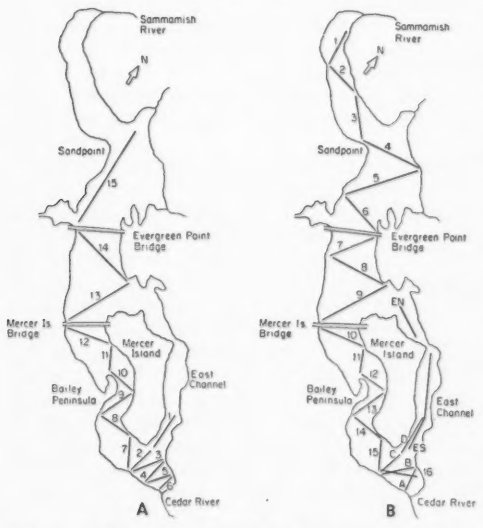

FIG. 1. Distribution of transects over Lake Washington during A) September 12 survey; B) November and December surveys. Transects 16, ES, and EN run only in December; transects A–D run only in November.

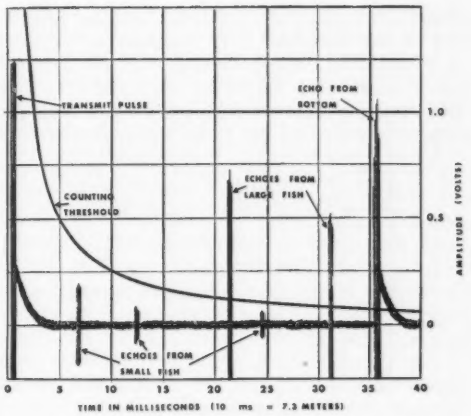

FIG. 2. Oscilloscope trace showing threshold for separating large and small fish targets.

passed over the fish during the September series. Then the average diameter of the echo sounder beam was determined from the relationship

$$d = \frac{4\ v\bar{p}}{\pi r},$$

where v is the boat speed in m/s, p is the average number of successive echoes, and r is the pulse repetition rate (two transmissions per second). Further description and discussion of the method are given in Thorne (1972) and Croker (1973).

For each transect, the number of large fish per square meter of surface area was determined by dividing the average number of echoes per pulse by the sampling area of the echo sounder. Total numbers were determined from the surface areas represented by the transects. The surface area associated with depths greater than 30 ft (9 m) was assumed for all transects.

Results and discussion — A total of 725 echoes on 576 targets was counted during the 15 transects on September 12, an average of 1.26 echoes per target, corresponding to an average sampling area of the echo sounder of 6.3 m^2. A total of 199 echoes was counted during the November series and 92 echoes in December. Densities of large fish observed in various sections of the lake and estimates of total large fish for the three series are in Table 1. Total estimates were 266,700 for September, 102,900 for November, and 50,200 for December.

A decrease of 163,800 large fish was observed between the September and November series. This difference compares well with a weir count up the

Cedar River of about 156,000 fish between September 12 and November 10 (J. Ames, WDF, personal communication). The December estimate of 50,200 fish represents an additional decrease of 52,700 fish; thus the total decrease was 216,500 fish. The total weir count up the Cedar River was 166,400 fish, of which 2500 passed before September 12. In addition there were an estimated 17,000 beach spawners and spawners in the Sammamish River and smaller streams. The total estimated escapement after September 12 was 181,000 — 35,500 less than the net decrease in acoustic estimates of large

TABLE 1. Densities of large fish in various sections of Lake Washington during September, November, and December surveys, 1971. Parentheses denote values assumed for unsampled areas.

Transect				Density (fish/10^3 m^2)		
Sept.	Nov.	Dec.	Section of lake	Sept.	Nov.	Dec.
			North lake			
	1	1			1.7	1.0
	2	2		(2.0)	0.3	0.6
	3	3			0.8	0
			Sand Point			
	4	4			1.3	0.1
15	5	5		2.0	1.3	0.5
	6	6			0.9	0.1
			Evergreen Point floating bridge			
	7	7			1.0	0
14				1.5		
			Central lake			
	8	8			1.8	0.4
13	9	9		1.3	1.0	0.5
			Lake Washington floating bridge			
12	10	10		0.8	0.1	0.7
11	11	11		1.6	0.7	1.0
			South lake			
10	12	12		1.8	1.6	1.5
9	13	13		9.3	0.8	2.5
8	14	14		12.0	2.9	1.2
7	15	15		14.4	2.9	0.7
			Cedar River vicinity			
6				10.3		
	A				3.6	
5				14.7		
4		16		10.9		2.7
	B				3.5	
3				11.1		
2	C			10.7	2.3	
			East Channel			
1	D	ES		4.8	1.7	0.4
		EN		(4.8)	(1.7)	0.6
Fish total				266,700	102,900	50,200

targets. The difference between the two estimates of escapement may be accounted for in several ways. It may indicate either mortality of adults in the lake or underestimation of the number of beach spawners and spawners in the Sammamish system. It may be due in part to the presence of other large fish in the lake. By September 12, about 66% of the approximately 15,000 chinook salmon run and about 10% of the approximately 35,000 coho salmon run would have been in the lake (S. Mathews, WDF, personal communication). By December 7, virtually all the chinook and coho salmon would have left the lake. Thus, these fish could account for 10,000–15,000 of the September 12 estimate. The estimates could have been affected by changes in the distribution of the resident large-size and intermediate-size fish during the 3 mo.

Results were also affected by sampling variability and error from several sources. The population estimates were directly affected by the estimate of the diameter of the echo sounder beam, which was based on measurements of durations in the sounder beam. Measurements of duration were made on all 576 targets insonified during the September 12 survey. The pulse repetition rate, depth of fish, and boat speed were such that most targets remained in the beam for only one transmission. The precision of the duration measurements could be increased by increasing the number of transmissions on fish, either by decreasing the boat speed or increasing the pulse repetition rate. A greater number of transmissions on each target would also aid the separation of intermediate-size targets. Other possible sources of error are measurement of boat speed, variation in depth distribution of fish, selection of the threshold level, and assignment of marginal targets.

Several procedural improvements are necessary. A standard set of transects should be devised reflecting the distribution of adults during full recruitment to the lake. The transects should cover all portions of the lake, provide replication so that sampling variance can be evaluated, and provide adequate sample size. The use of a standard transect series will facilitate direct comparison of data.

More precise measurement of the sampling volume, as well as improved separation of large and small fish, can be made by increasing the number of pulses on individual fish. The bottom of Lake Washington is shallow enough to allow a pulse repetition rate of four per second without interference from multiple bottom reflections.

The estimate of 267,000 large fish obtained on September 12 overestimates adult sockeye salmon. An estimate of the resident large fish and other adult salmon besides sockeye was obtained by comparing total estimate with weir counts and spawning ground surveys after the migration upriver. The magnitude of this component included in an estimate of large fish made during full sockeye salmon recruitment to the lake is essential if the result is to be of immediate value in the regulation of harvest. This problem may be minor if the non-sockeye salmon component is similar from year to year. In any case, it should be possible to estimate the size of the resident fish population by surveying in late May or early June before the adult sockeye enter the lake. If a correlation between rate of buildup and ultimate run size could be determined, it could provide a valuable early index of the run size.

Acknowledgments — Dr R. Burgner provided invaluable guidance during all aspects of this study. The cooperation of personnel of the Washington State Department of Fisheries is gratefully acknowledged. This study was supported by the Washington Sea Grant Program under the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, NOAA Grant No. 1-35320.

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Stream Migration of Yearling Splake (*Salvelinus fontinalis* × *S. namaycush*) Planted in Georgian Bay[1]

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BERST, A. H., AND N. R. PAYNE. 1974. Stream migrations of yearling splake (*Salvelinus fontinalis* × *S. namaycush*) planted in Georgian Bay. J. Fish. Res. Board Can. 31: 226–229.

Yearling splake (*Salvelinus fontinalis* × *S. namaycush*) (F_4, F_5) released into Georgian Bay in 1971 and 1972, when lake surface temperatures averaged 5.5 C lower than the hatchery temperature, tended to move into streams within 3.6 km of the planting site where temperatures approached the hatchery temperature and the temperature preferendum of the hybrid. This tendency was not apparent in splake planted at later dates when the lake and hatchery temperatures were similar. Varying stream flows had no discernible effect on the migration of splake into the study streams.

BERST, A. H., AND N. R. PAYNE. 1974. Stream migrations of yearling splake (*Salvelinus fontinalis* × *S. namaycush*) planted in Georgian Bay. J. Fish. Res. Board Can. 31: 226–229.

Des truites moulac (*Salvelinus fontinalis* × *S. namaycush*) de moins d'un an (F_4, F_5) introduites dans la baie Georgienne en 1971 et 1972 au moment où la température de surface du lac était en moyenne de 5.5 degrés C inférieure à celle de l'établissement de pisciculture eurent tendance à pénétrer dans les cours d'eau en dedans de 3.6 km du lieu d'ensemencement, là où la température se rapprochait davantage de celle de la pisciculture et de la température préférentielle de l'hybride. Cette tendance n'a pas été apparente chez des truites moulac ensemencées plus tard, quand les températures du lac et de la pisciculture étaient les mêmes. Les variations du débit des cours d'eau n'eurent pas d'effet notable sur les migrations de la truite moulac dans les cours d'eau sous étude.

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SINCE 1969, annual plantings of splake for the trout rehabilitation of Lake Huron have been made in Lake Huron and Georgian Bay by the Province of Ontario. The hybrids have been the progeny of 3rd and 4th generation parents selected for early maturity and for the ability to occupy deep water (Tait 1970; Berst and Spangler 1970).

Soon after plantings of splake were made in the Meaford area of Georgian Bay (44°36′N, 80°35′W) and the Douglas Point area of Lake Huron (44° 19′N, 81°36′W) field staff observed significant numbers of planted hybrids in the lower sections of streams up to 22 km from the release sites (D. P. Dodge and G. Harris personal communication, Ontario Ministry of Natural Resources). Concentrations of splake in streams, where they are more subject to angling, illegal netting, relative scarcity of food, and predation by birds and mammals, are likely to sustain heavier losses than in the lake. As a high survival of the planted hybrids to sexual maturity at age III is considered important to the establishment of self-sustaining stocks, such losses in streams should be avoided if possible. To this end, information is needed on the movement of splake in and out of streams and on factors which influence this behavior.

This report describes an experiment in which plantings were made in two series about a month apart in 1971 and 1972 to study the movement of planted splake into streams in relation to lake and stream temperatures and stream flow.

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Methods — Releases of marked (fin-clipped) yearling splake were made near shore in the planting area (Fig. 1) in the spring of each year as follows:

Year	Period	Mean no. fish per kg	No. planted
1971	Apr. 19–30	16.8	93,045
	June 3–4	13.9	9,095
1972	May 4–5	13.2	20,600
	June 6–16	18.1	34,465

Tributary streams within 5 km of the planting area were periodically checked for the presence of splake during 3 wk following the plantings. In streams where splake were present, a portable AC–DC backpack fish shocker (MacCrimmon and Berst 1963) was used to estimate the areal distribution and number of splake. On some dates specimens were removed from the streams for examination of stomach contents. Flow rates and water temperatures were recorded for streams in the study area as well as lake temperatures in the vicinities of stream mouths. The second series of plantings in each year included 886 and 591 splake, respectively, which had been marked with streamer tags attached by means of monofilament nylon through the anterior base of the dorsal fin.

The study streams were inspected for the presence of splake in late October and early November in each year after the stream temperatures had returned to the May–June levels (7–15C).

Observations — On May 3, 1971, 3 days after the initial series of releases, planted splake were observed in streams A and B, 2.0 km and 3.6 km from the planting site, respectively. On May 5, use of the shocker showed the splake were present in streams A and B for distances of 80 and 205 m from the mouth, respectively, in numbers estimated at 63 and 400. Subsequent inspections of these streams on May 17, June 8, and June 15, 1971 showed numbers in A of 60, 15, and 18 and in B of 250, 40, and 22, respectively (Fig. 2).

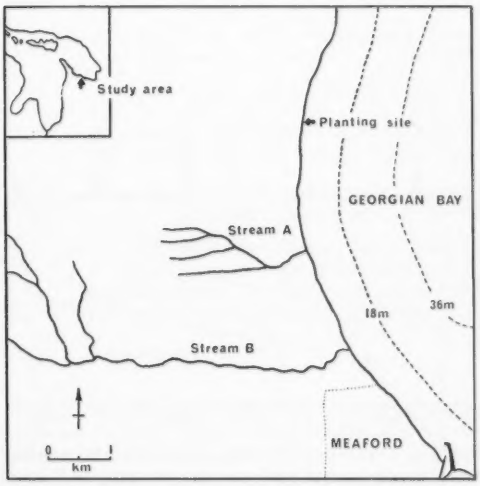

FIG. 1. Study area, planting site, and study streams.

In 1972, the first inspection was made May 11, 6 days after completion of the first series of releases. It revealed similar distributions of splake in the two streams with an estimated 10 hybrids in stream A and 64 in B. Repeat inspections on June 14, June 28, and July 10 showed the numbers in A of 11, 6, and 4 and in B of 32, 36, and 2, respectively.

There was no appreciable interruption in the decline of the stream populations of splake following the second series of plantings in either year of the study. Of the tagged splake released as part of these later plantings, only two were recovered in streams, both in 1972 in stream B within 12 days of planting. No comparable effort was made to recover tagged splake from Georgian Bay.

Personal observations and reports from residents indicated anglers removed several hundred splake from stream B in 1971, but most of this exploitation occurred prior to the initial survey using the shocker. The estimate of 400 hybrids is, therefore, probably well below the total hybrids entering the stream from the first series of plantings made in that year. With fewer splake available in stream B in 1972, it held little attraction to anglers, and for the same reason, only token fishing occurred in stream A over the 2 yr.

In 1971 and 1972, lake surface temperatures in the study area during the first series of plantings averaged 2–3 C, which was 5.5 degrees lower than the hatchery temperature (Fig. 2). Study stream temperatures at the same time averaged 7 C, only 1 degree lower than the hatchery temperature. During the second series of plantings in 1971, the surface temperature of the lake was identical (8.5 C) to the hatchery temperature, while those of the streams averaged about 6.5 degrees higher than that of the hatchery. At the time of the second series of plantings in 1972, the lake surface temperatures averaged 2 degrees lower (6.5 C) and the stream temperatures approximately 4.5 degrees higher (13 C) than that of the hatchery. By mid-June stream temperatures had risen to approximately 17 C in 1971 and to 15 C in 1972.

Flow rates in streams A and B in 1971 declined from 140 to 56 and from 380 to 127 liters/s, respectively, over the time inspections were made. In 1972, they declined from 95 to 31 liters/s in stream A and from 340 to 119 liters/s in B.

The hybrids observed in the streams in each year were in apparently healthy condition. Specimens for laboratory examination were taken as follows:

	Stream A	Stream B
May 5, 1971	28	35
May 17, 1971	–	107
May 11, 1972	10	14

Lengths of these splake showed them to be representative of the planted fish. There was no apparent relationship between the relative size of hybrids and the distance they moved upstream. Stomachs of fish in the samples were empty, except for 16 which contained invertebrates.

The stream inspections in October and November of each year revealed no splake in either stream.

Discussion and conclusions — Splake planted from late April to early May, when water was 5 degrees or more below hatchery temperatures, tended to move into the lower sections of nearby streams where temperatures approximated the temperature to which they had been acclimated in the hatchery (8 C) and the thermal preferendum for F_1 splake of 12 C (Pearson 1952). Splake did not move into streams in any numbers when planted in early June, after lake temperatures had risen to 8 C or higher. As a consequence of releasing proportionately more splake later in the season in 1972 than in 1971, a significantly smaller percentage from the 1972 planting entered the streams.

Klein (1966) found that splake moved from Parvin Lake into an inlet stream when the surface temperature of the lake was 16–19 C and the stream temperature was 3–13 C. Although the movement of the fish in Klein's study was from warm to cold water it still represents movement in the direction of the hybrid's preferendum. In this respect, the findings of the two studies are in agreement.

Flow rates in stream B following the second series of plantings were comparable to, or greater than, those in stream A following the first series of plantings in both years. Since the numbers of splake which entered stream B following the second series of plantings in both years were insignificant, stream flow was not a major factor in attracting

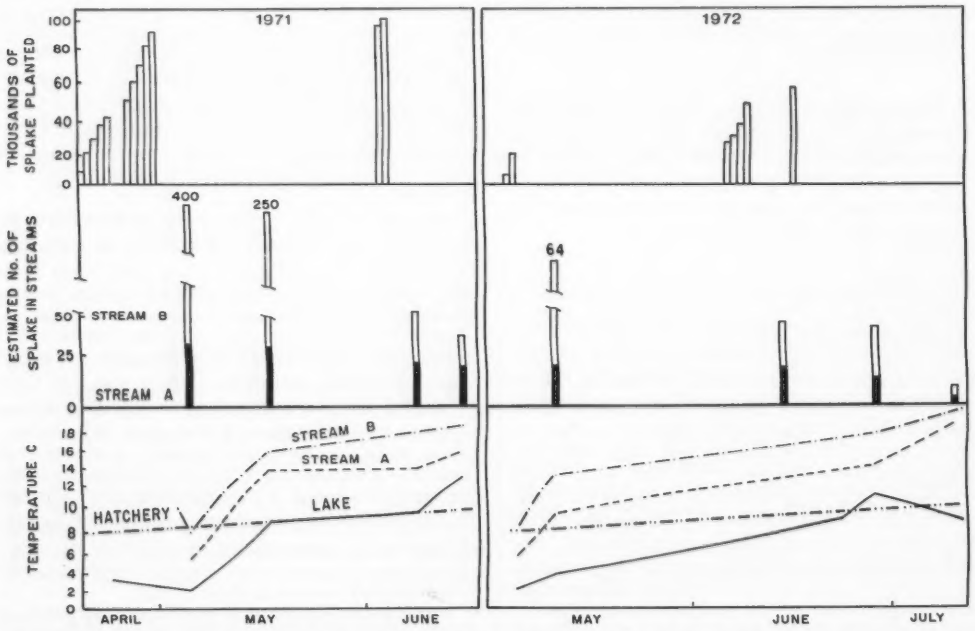

FIG. 2. Cumulative numbers of splake planted in the Meaford area in 1971 and 1972 (top panel), estimated numbers in the two streams in each year (center panel), and surface water temperature of study streams, hatchery, and lake in each year (bottom panel).

splake into the streams. This conclusion is further supported by the fact that a rapid rise in stream flows following heavy rains on June 22, 1972 resulted in no apparent increase in the number of hybrids in either stream.

Although a high level of exploitation of stream migrants by anglers occurred early in the season, when splake were concentrated in the lower section of stream, angling pressure in both streams was insignificant after early May as the numbers in the two streams declined. In view of this and in the absence of observed mortalities of splake, we conclude that the majority of hybrids not collected for laboratory examinations returned to the lake. The apparent movement of splake from the streams to the lake was presumably in response to the warming of stream temperatures above the preferred temperature of the hybrids. The apparent absence of splake from the survey streams in the fall, when stream temperatures had fallen to tolerable limits, suggests that the stream environment held no special attraction to splake which had acclimated to lake conditions. Also, no mature splake appeared in either stream in the fall of 1971 or 1972 even though a limited gillnetting effort by commercial fishermen produced several from shoals in southern Georgian Bay at those times.

The movement of planted splake into streams can be substantially reduced by releasing them when lake suface temperatures are within 2 or 3 C of hatchery temperatures.

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Feeding by Alewives (*Alosa pseudoharengus*) on Larval Lake Whitefish (*Coregonus clupeaformis*) in the Laboratory[1]

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HOAGMAN, W. J. 1974. Feeding by alewives (*Alosa pseudoharengus*) on larval lake whitefish (*Coregonus clupeaformis*) in the laboratory. J. Fish. Res. Board Can. 31: 229–230.

Adult alewives (*Alosa pseudoharengus*) from Lake Michigan readily ate larval lake whitefish (*Coregonus clupeaformis*) of 16.3 mm (standard deviation 1.4 mm) average length in the laboratory. They ignored the larvae when the latter had grown to 19.5 mm (SD = 1.6 mm) and 24.9 mm (SD = 3.2 mm) average length.

HOAGMAN, W. J. 1974. Feeding by alewives (*Alosa pseudoharengus*) on larval lake whitefish (*Coregonus clupeajormis*) in the laboratory. J. Fish. Res. Board Can. 31: 229–230.

Des gaspareaux (*Alosa pseudoharengus*) du lac Michigan ont mangé volontiers des larves de grand corégone (*Coregonus clupeaformis*) d'une longueur moyenne de 16.3 mm (écart type de 1.4 mm). Ils n'en tinrent aucun compte alors que les larves avaient atteint des longueurs moyennes de 19.5 mm (écart type = 1.6 mm) et 24.9 mm (écart type = 3.2 mm).

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SINCE the alewife spread to the Great Lakes in the late 1940s (Miller 1957) it has had pronounced effects on the fish fauna (Smith 1970; Brown 1972) and zooplankton (Wells 1970). Its contribution to the ecosystem is both detrimental and beneficial. This note documents the experimental feeding of *lake whitefish larvae to alewives.*

Larval lake whitefish were offered to alewives (130–180 mm, age III–V) on three occasions in 1970: on May 21, in a wading pool 3.4 m × 3.4 m × 30 cm at 17.0 C (five lots of 10 larvae each in succession); on June 9, in a rectangular tank at 12.0 C and in the wading pool at 16.0 C (10 larvae each); and on June 25, in the wading pool at 17.9 C (10 larvae). Average lengths of the larvae were: 16.3 mm with an observed standard deviation of 1.4 mm (3–4 wk old), 19.5 mm (SD = 1.6 mm), and 24.9 mm (SD = 3.2 mm).

On May 21, the larvae were nearly transparent, not yet silvery, full-bodied, or very different from the typical "pinhead" shape of many larval fishes. On June 9, the sides of the larvae were beginning to show the characteristic silver opaqueness of whitefish fry and their dorsal surfaces were becoming dark. On June 25, the larvae (fry) had adult proportions and coloration.

The alewives were captured in the Milwaukee harbor, held in wading pools at 17.0 C, and fed on live and frozen *Daphnia* spp. On the three dates, and in the two tests on June 9, different groups of 12 alewives were used. In all tests, the alewives swam at a slow pace in a loose school.

On May 21, the alewives ate all the larvae offered. They began feeding on the first lot after 3 min and ate all the larvae within 8 min, ate all the second lot within 2 min, and ate all the fifth within 44 s.

In the later trials, the alewives ate none of the larvae. On June 9, in both the pool and the tank, the alewives approached the larvae as if to swallow them but turned away at 5–15 cm and ceased their attack behavior after several minutes. On June 25, they ignored the young whitefish.

On the basis of these tests, alewives could not prey appreciably on whitefish larvae in nature. Whitefish larvae in Green Bay and adjacent Lake Michigan grow to 17–22 mm in shallow water before the onshore movement of the alewives in May–June (Hoagman 1973). Also, the whitefish rebounded to high abundance when the alewife was most abundant from 1964 to 1970 (Walter and Hoagman 1971; Brown 1972).

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Rarely Seen Fishes Captured in Hudson Submarine Canyon

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HAEDRICH, R. L., AND P. T. POLLONI. 1974. Rarely seen fishes captured in Hudson Submarine Canyon. J. Fish. Res. Board Can. 31: 231–234.

The alepocephalid *Rouleina mollis* (Koehler 1896), the ophidiid *Xyelacyba myersi* Cohen 1961, and the two macrourids *Nezumia longebarbatus* (Roule and Angel 1933) and *Sphagemacrurus grenadae* (Parr 1946) were trawled in Hudson Submarine Canyon (ca. 39°18′N 72°00′W) between 1500 and 1960 m on the bottom. These records constitute considerable extensions of the known ranges. The eretmophorid *Halargyreus johnsoni* Günther 1862, previously recorded from Hudson Canyon but unknown elsewhere in the area, was also taken. The trawl haul which captured these noteworthy species took, in addition, 290 specimens in 18 species of deep benthic fishes. Abundant species included *Antimora rostrata, Synaphobranchus kaupi, Alepocephalus agassizi, Nezumia bairdii, Dicrolene intronigra,* and *Aldrovandia phalacra.* These species also predominated in a catch made in a similar haul at the same place 4 yr previously, suggesting that the species composition of such deep-living canyon assemblages is quite stable.

HAEDRICH, R. L., AND P. T. POLLONI. 1974. Rarely seen fishes captured in Hudson Submarine Canyon. J. Fish. Res. Board Can. 31: 231–234.

L'alépocéphalide *Rouleina mollis* (Koehler 1896), l'ophidiide *Xyelacyba myersi* (Cohen 1961) et les deux macrourides *Nezumia longebarbatus* (Roule et Angel 1933) et *Sphagemacrurus grenadae* (Parr 1946) ont été capturés au chalut de fond dans le caynon sous-marin de l'Hudson (ca. 39°18′N 72°00′W) à des profondeurs de 1500 à 1960 m. Ces mentions représentent une extension considérable de l'aire. L'éretmophoride *Halargyreus johnsoni* (Günther 1862), déjà signalé dans le canyon de l'Hudson mais inconnu ailleurs dans la région, a été également capturé. Le trait de chalut qui a rapporté ces espèces dignes de mention a de plus donné 290 spécimens appartenant à 18 espèces de poissons bathybenthiques. Les espèces les plus abondantes incluent *Antimora rostrata, Synaphobranchus kaupi, Alepocephalus agassizi, Nezumia bairdii, Dicrolene intronigra* et *Aldrovandia phalacra.* Ce sont ces mêmes espèces qui dominaient dans un trait de chalut semblable au même endroit, 4 ans auparavant, ce qui donne à croire que la composition spécifique des populations habitant les profondeurs du canyon est assez stable.

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As part of a study of the ecology of the epibenthic communities in submarine canyons, bottom trawling was conducted in Hudson Canyon (ca. 39°30′N, 72°20′W) during February and March 1973. Included in the catches were several species known hitherto from only a very few specimens taken in localities far removed from the New York Bight. Virtually all the unusual species were taken in a single deep collection made with a 12.2-m (40-ft) shrimp trawl, providing convenient material for a comparison with another trawl collection made in almost the same position 4 yr previously (Haedrich and Horn 1970).

Species of particular interest include the alepocephalid *Rouleina mollis*, the eretmophorid *Halargyreus johnsoni*, the ophidiid *Xyelacyba myersi*, and the two macrourids *Nezumia longebarbatus* and *Sphagemacrurus grenadae*. The specimens are deposited in the United States National Museum. All were taken in haul 216, at 39°16.5′N, 71°58.0′W to 39°18.5′N, 72°01.0′W, 25 February 1973, time on bottom ca. 1335–1435 h, depth 1500–1960 m, with a 12.2-m (40-ft) Gulf of Mexico shrimp trawl, R/V *Chain* cruise 111. The gear was fished up the axis of Hudson Canyon, and took over 400 kg (1000 lb) of heavy grey clay and over 50 kg (several hundred pounds) of gravel and pebbles.

Three specimens of the alepocephalid *Rouleina mollis* Koehler 1896, 230–320 mm SL, were taken. These are conspecific with the specimens referred by Haedrich and Horn (1970) to *Rouleina sp.* The lack of scales is said to distinguish the genus (Parr 1951), but close examination revealed that scales are in fact present but only on the lateral line, appearing as barely discernible incomplete rings around the pores. Our specimens agree very

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well with the descriptions given by Koefoed (1927) and by Roule (1919) for *Talismania mollis* (= *Bathytroctes mollis* Koehler). Important features are the smooth black skin, the large mouth, the moderately large eye located high on the head, the maximum depth of the body at the neck, and especially the low number (six) of pectoral finrays. Parr (1951) considered *R. mollis* a junior synonym of *R. attritus* Vaillant 1888. Vaillant's battered specimen, however, is described as having scales, and comparison of the otoliths from our material with those in Vaillant's figure (1888, pl. XII, 2) revealed significant differences. Roule's (1919) single specimen was from the Azores, and Koefoed's (1927) 18 specimens were taken off Morocco. We have seen what appear to be the same species, but identified as *R. aequatoris*, in Gulf of Mexico collections housed in the USNM. Counts and measurements are: standard length 230, 235, 310 mm, head length 74, 75, 100 mm, snout 23.3, 22.2, 31.0 mm, eye diameter 13.6, 14.5, 17.8 mm, length of upper jaw 41.3, 41.5, 52.3 mm, interorbital width 13.0, 13.5, 19.4 mm, pectoral fin length –, 23.5, 31.3 mm, pelvic fin length –, –, 25.1 mm, predorsal distance 154, 150, 206 mm, preanal distance 151, 150, 199 mm, maximum depth 42.7, 46.0, 57.5 mm, depth caudal peduncle 15.0, 16.7, 23.3 mm, dorsal finrays 17, 18, 19, anal finrays 17, 18, 19, pectoral finrays 6, 6, 6, pelvic finrays 7, 7, 7, gill rakers (first arch) 5+1+18, 5+1+16, 6+1+15, vertebrae 46, 46, 46.

Two specimens of the eretmophorid *Halargyreus johnsoni* Günther 1862, 236 mm and 318 mm SL, were taken. This antitropical species (Cohen 1974) appears to have one center of distribution in the northeastern Atlantic, but is known in three specimens from the Grand Banks (Templeman 1968) and three from Hudson Canyon (Haedrich and Horn 1970). Since 1970, we have sampled the slope to the east of Hudson Canyon fairly extensively, but have taken no *Halargyreus*, suggesting that this species may live, in our area, only in large canyons.

Our two specimens of the ophidiid *Xyelacyba myersi* Cohen 1961, 140 and 340 mm SL, are the fourth and fifth recorded in the literature. The holotype and two other specimens were taken by MV *Oregon* between 850 and 1100 fm in the Gulf of Mexico during 1959–60. Our specimens agree in all respects with the original description; diagnostic characters are the heavy spines on the opercle and preopercle, the short snout lacking nasal spines, a terminal mouth, and the posterior nostril a prominent pit immediately before the eye. Counts and measurements for the two specimens (smallest first) are: dorsal finrays –, 88, anal finrays –, 74, pectoral finrays 18, 18, pelvic finrays 2, 2, vertebrae (excluding hypural) 49, standard length 141, 340 mm, greatest depth 37, 111 mm, depth at vent 33.5, 100 mm, greatest width of head 21.4, 55.5, width at vent 10.5, 34.2 mm, preanal 51.1, 136 mm, preventral 18.7, 46.5 mm, length of pectoral fin 16 (broken), 58.6 mm, length of pelvic fin 21.9, 65, base of pectoral fin 11.4, 28.2 mm, head length (excluding spine) 31.5, 75.7 mm, snout 11.0, 27.3 mm, eye diameter 3.4, 6.8 mm, length of upper jaw 18.9, 48 mm, greatest depth of maxillary 6.5, 18.5 mm.

Our 407-mm specimen of the macrourid *Nezumia longebarbatus* (Roule and Angel 1933) is the third known. The holotype was taken in 1700 m south of Madeira in 1911, and a second specimen by M/V *Oregon* at 1460 m in the Gulf of Mexico during 1970 (Marshall and Iwamoto 1973). This species is most readily distinguished from its congeners by the long barbel and the high number (13) of pelvic rays. Counts and measurements are: total length 386 mm, head length 68 mm, length of snout 31% of head, ventral length of snout 29%, snout to first dorsal 118%, snout to anal fin 153%, diameter of orbit 28%, interorbital width 19%, length of premaxilla 26%, length of barbel 25%, depth of body at first dorsal 92%, height of first dorsal 83%, length of pectoral 59%, length of pelvic 48%, first dorsal finrays 10, pectoral finrays 21, pelvic finrays 13, scales below first dorsal 9.

Our specimen of the macrourid *Sphagemacrurus grenadae* (Parr 1946) is the ninth known, and the first outside the Caribbean–Gulf of Mexico area. The holotype was taken off Grenada, British West Indies, at 1050 m; a second specimen was taken by M/V *Oregon* at 1000 m off Nicaragua (Bullis and Thompson 1965), and Marshall and Iwamoto (1973) report six additional specimens from ca. 890 m off Panama in the Caribbean and two from ca. 1040 m in the Straits of Florida. The position of the pelvic fins, well forward of the pectorals, and the peritroct just before the anal fin help distinguish this species. Counts and measurements are: total length at least 204 mm (broken), head length 34 mm, length of snout 31% of head, snout to first dorsal 127%, snout to second dorsal 194%, snout to anal fin 144%, snout to pelvic fin 98%, diameter of orbit 31%, interorbital width 25%, length of premaxilla 29%, length of mandible 34%, length of barbel 15%, depth of body at first dorsal 97%, first dorsal finrays 11, pectoral finrays 20, pelvic finrays 12.

The capture of two *Lampanyctus crocodilus* (Myctophidae), 105 and 116 mm SL, in the trawl is noteworthy because this species is generally considered mesopelagic. When young, it certainly is,

but as an adult it may well live on or near the bottom. Dr J. Craddock (personal communication) has observed large individuals of this species not infrequently on the continental slope south of New England during dives in the DSRV *Alvin*.

In addition to the specimens described above, haul 216 took 288 individuals in 17 species of deep benthic fishes. Included were: one *Raja bathyphila* 454 mm TL, three *Venefica procera* 473–746 mm SL, 29 *Synaphobranchus kaupi* 245–670 mm SL, five *Aldrovandia affinis* 115–465 mm SL, 21 *Aldrovandia phalacra* 235–470 mm SL, five *Halosauropsis macrochir* 160–710 mm SL, 17 *Alepocephalus agassizi* 178–445 mm SL, one *Alepocephalus productus* 400 mm SL, one *Bathysaurus agassizi* 167 mm SL, three *Bathypterois phenax* 168–180 mm SL, 154 *Antimora rostrata* 165–490 mm SL, 21 *Dicrolene intronigra* 195–306 mm SL, two *Lycenchelys paxillus* 303–312 mm SL, one *Melanostigma atlanticum* 98 mm SL, one *Coryphaenoides rupestris* 900 mm SL, 10 *Lionurus carapinus* 68–195 mm SL, and 13 *Nezumia bairdii* 263–440 mm SL.

On March 6, 1969 during R/V *Chain* cruise 88, we made a haul to about 1280 m on the south slope of Hudson Canyon with a 12.2-m (40-ft) Gulf of Mexico shrimp trawl. The trawl captured 518 individuals in 24 species of deep benthic fishes, including three *Halargyreus johnsoni*. The occurrence of these three in Hudson Canyon constituted a significant extension of the known range for the species, and was so reported (Haedrich and Horn 1970). The haul was made at a position (39°09.2′N, 72°11.5′W) but little removed from that for haul 216. Thus, the two hauls were made in about the same position and the same season; the major differences between them are that haul 216 fished somewhat deeper than haul 7, haul 216 was in the canyon axis whereas haul 7 was along the south wall, and haul 216 was removed 4 yr in time from haul 7.

Table 1 shows the rank abundance of the six top-ranking species from each haul. *Antimora rostrata* and *Synaphobranchus kaupi* ranked first and second, respectively, in both. Thereafter, agreement was rather close, involving mostly the same species with only a few step differences in rank. These minor differences may be related to the fact that the hauls were made at slightly different depths. *Nezumia bairdii*, for example, decreases in abundance below 1000 m (Marshall and Iwamoto 1973; R. L. Haedrich unpublished data), whereas *Aldrovandia phalacra* has its center of abundance generally below this depth (McDowell 1973; and R. L. Haedrich unpublished data). The shifts in rank shown in Table 1 are as would be expected in the light of such knowledge. Overall, the compositions of the two collections are considerably alike (percent similarity by the formula of Whittaker and Fairbanks (1958) = 60.5%). One of the most often cited attributes of the deep ocean environment is its assumed stability (Sanders 1968). Even such a small sample as ours supports this contention.

TABLE 1. Ranks and (in parentheses) numbers of the six top-ranking species in trawl hauls 7 and 216.

	Haul 7	Haul 216
Antimora rostrata	1(141)	1(154)
Synaphobranchus kaupi	2(71)	2(26)
Alepocephalus agassizi	3(67)	5(17)
Nezumia bairdii	4(63)	6(11)
Dicrolene intronigra	5(52)	3.5(21)
Lionurus carapinus[a]	6(49)	7(10)
Aldrovandia phalacra	9(6)	3.5(21)
Total species	24	23
Percent similarity	60.5%	

[a]This species, represented largely by small battered specimens, was included by Haedrich and Horn (1970) in "*Macrourus bairdii*." The availability of up-to-date keys (Marshall and Iwamoto 1973) has allowed proper reidentification.

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A Comparison of the Storage Life and Bacteria Counts of Chilled Cod Fillets Packaged in Wooden Boxes and Heat-Sealed Plastic Containers

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Codfish held for 4 days on ice on board a dragger were filleted and the fillets were placed in two types of containers: (a) parchment-lined wooden boxes, (b) hermetically sealed plastic boxes. The boxes were stored at 0 C (32 F) for up to 8 days. Samples withdrawn at day 0, 4, 7, and 8 were analyzed for trimethylamine content and assessed for quality. The samples were also analyzed for total bacteria, total yeast and molds, lactobacilli, and anaerobic and facultative anaerobic spore- and nonspore-forming bacteria. The results indicated that the fish flesh had a total storage life of 10–11 days, regardless of packaging, and that the storage temperature of 0 C (32 F) was too low for the growth of anaerobic spore-forming bacteria.

VARGA, S., W. A. HIRTLE, AND W. E. ANDERSON. 1974. A comparison of the storage life and bacteria counts of chilled cod fillets packaged in wooden boxes and heat-sealed plastic containers. J. Fish. Res. Board Can. 31: 234–237.

Nous avons découpé en filets des morues gardées dans la glace pendant 4 jours à bord d'un petit chalutier, et placé les filets dans deux types de récipients: (a) boîtes en bois tapissées de parchemin à l'intérieur et (b) boîtes en plastique fermées hermétiquement. Nous avons entreposé les boîtes à 0 C (32 F) pendant des périodes allant jusqu'à 8 jours. Nous avons prélevé des échantillons aux jours 0, 4, 7 et 8, sur lesquels nous avons fait des dosages de triméthylamine et des tests de qualité. Sur ces échantillons, nous avons également analysé les bactéries totales, levures et moisissures totales, lactobacilles, bactéries anaérobies strictes ou facultatives, sporulantes ou non. Les résultats démontrent que la chair du poisson a une durée de conservation de 10–11 jours, quelle que soit la méthode d'emballage, et que la température d'entreposage de 0 C (32 F) est trop basse pour permettre la croissance de bactéries anaérobies sporulantes.

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FRESH fillets were traditionally shipped from the east coast of Canada to inland market in wooden boxes. These boxes were placed in wooden master containers and refrigerated with ice (ice:fillet ratio 2:1). The processing companies and transporting firms recently modified this shipping practice. The new shipping method consists of packing the fillets 4.5 kg (10 lb) into hermetically sealed plastic boxes and icing these boxes while packing in waterproof master containers.

Alterations in packaging procedure could cause change in storage life, spoilage pattern, and health hazard of foods, which are of concern to industry and controlling agencies. Thus, this study was undertaken to determine whether changes, if any, occur in spoilage pattern and bacteria flora of chilled fillets as a consequence of altered packaging method.

Materials and methods — Codfish (*Gadus morhua*) were caught by a dragger operating off the eastern shore of Cape Breton Island on April 27, 1972. The fish (90.9 kg (200 lb)) was dressed immediately after catching and was held for 4 days on ice in the hold of the vessel. After landing the fish was filleted in the fish plant, packaged, and iced, then shipped to the Fish Inspection Laboratory (Halifax) on May 1, 1972.

Fillets were packaged in two different ways. In Treatment 1, 4.5 kg (10 lb) were placed in parchment-lined wooden boxes (31.8 × 19.7 × 7.6 cm) and in Treatment 2, 4.5 kg were placed in plastic boxes (26.7 × 19.1 × 12.1) obtained from General Plastics Ltd., Cookshire, P. Q. The plastic boxes (Specification No. 61470) were made from special-brand polyethylene. The filled plastic boxes were heat-sealed hermetically.

The boxes of fillets were maintained at 0 ± 0.5 C in a mechanically cooled storage cabinet. Each box was surrounded with flaked ice to ensure alternate refrigeration in the event of mechanical failure. The temperature of the fillets was monitored constantly by calibrated copper-constantan thermocouples to an accuracy of ± 0.1 C.

At each sampling day (0, 4, 7, and 8 days) fillets were examined from previously unopened container.

The concentration of trimethylamine (TMA) in cod flesh stored on ice was shown by Hoogland (1958) to correlate well with the eating quality of cod flesh. Thus, the TMA was determined in samples by the picrate method of Dyer (1945, 1959). The concentration of trimethylamine nitrogen was expressed as "mg %." Fillets for chemical examinations were taken randomly from the sample box. Sections totalling 100 g from two ends and middle parts of the fillets were used for each TMA determination.

Taste panel assessments were carried out by a trained taste panel. Panelists were selected from the staff of the laboratory. They were trained and the samples assessed on the basis of standards developed by Varga (1971). Each panel score represents the average of eight independent judgements. Overall quality is expressed by the lowest score obtained on either the appearance, odor, flavor, or texture of the sample. A score of 4.5 refers to the following conditions of the sample: (a) appearance: dark, greyish, yellowing, greenish, (b) odor: slight ammonia, fishy, (c) flavor: fishy, bitter after taste, (d) texture: soft, or dry and tough. A score of 10 denoted the most favorable and a score of 0 denoted the least favorable assessment. With a panel score of $\lesssim$4.5 on any quality characteristics the sample was considered unfit as food. A taste panel assessment was carried out on each sampling date. A sample (1 kg (2 lb) randomly taken fillets) was baked for 25 min 170.1 C (450 F) in foil-covered aluminum pans. Prior to serving each of the pans were coded with random numbers to preclude biased assessment. Odor was rated by individual panelists before the volatile compounds had time to dissipate. Appearance, flavor, and texture were rated individually on 57–85 g (2–3 oz) portions.

TABLE 1. Mean taste panel scores and trimethylamine values at different storage times of cod fillets subjected to two treatments.

	Treatments			
	Wooden box		Plastic Box	
Storage time of fillets in days (0 C)	Mean taste panel score	Mean[a] TMA (*mg N/100 g*)	Mean taste panel score	Mean[a] TMA (*mg N/100 g*)
0	8.4	1.27(0.21–3.60)	8.4	1.27(0.21–3.60)
4	6.0	4.24(3.60–5.30)	7.0	4.03(2.33–4.88)
7	3.5	28.8(16.5–42.4)	3.3	30.0(24.4–33.9)
8	1.8	>42.4	2.3	>42.4

[a]Each TMA mean represents three determinations; range in parentheses.

The total bacteria count was obtained for each sample as follows: a 100-g sample was transferred to a sterile Waring blendor jar. The sample was then blended for 2 min with 200 g of sterile peptone water (0.1% w/v). The homogenate was diluted with sterile peptone water and an aliquot was placed on (10 ml) tryptone glucose yeast extract agar (peptone 0.5% w/v, tryptone 0.5% w/v, yeast extract 0.25% w/v, glucose 0.1% w/v, sodium chloride 0.5% w/v, agar 1.5% w/v, and water). The plates were incubated at 25 C for 72 h. The total count per gram of sample was derived by multiplying the count obtained with the Quebec colony counter, by the appropriate dilution factor.

The total mold and yeast count was obtained on Bacto (BB) potato dextrose agar (tartaric acid added 10% w/v, 1.5 ml/100 ml medium), and lactobacillus count on Bacto (BB) tomato juice agar. These counts were derived similarly to that of total bacteria counts.

The count of anaerobic and facultative anaerobic spore and nonspore-forming bacteria was obtained from each sample as follows: an aliquot (1.0 ml) of the appropriate dilution of the same homogenate as was prepared for total bacteria count was inoculated into a set of MPN tubes which contained freshly boiled cooked meat enrichment medium. The contents of the tubes were mixed and sterile mineral oil layered over the surface of the culture. The tubes were then incubated for 7 days at 25 C. The cooked meat enrichment medium consisted of 2 g cooked meat and 5 ml enrichment solution made up from glucose (0.5% w/v), soluble starch (0.2% w/v), beef extract (1.8% w/v), yeast extract (0.5% w/v), and water. Cultures from the tubes which showed growth were streaked on reinforced

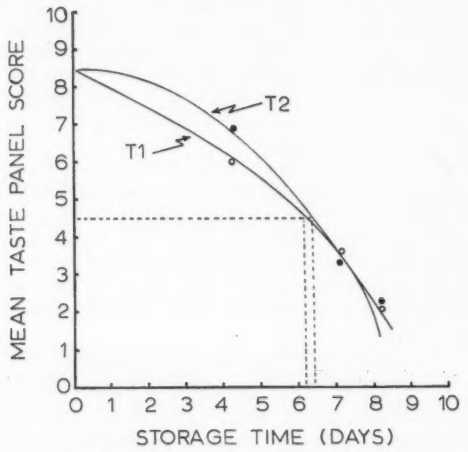

FIG. 1. Relationship of mean taste panel score and storage time at 0–C for fillets subjected to various treatments. T1, open circles, packaged in wooden boxes; T2, closed circles, packaged in heat-sealed plastic containers.

TABLE 2. Results of bacteriological analyses of fillets packaged in A) wooden boxes, B) heat-sealed plastic containers and stored for various time periods.

Type of Analysis	Treatment	Count[a] 0 day	4 day	8 day
Total plate count	A	375,000	980,000	1,230,000
	B	–	318,000	637,000
Yeast and mold count	A	24,000	29,000	8,000
	B	–	15,000	15,700
Lactobacillus count	A	279,000	460,000	990,000
	B	–	104,000	410,000
MPN estimate of anaerobic and facultative anaerobic nonspore-forming bacteria	A	460	1,100	460
	B	–	4,600	460
MPN estimate of anaerobic and facultative anaerobic spore-forming bacteria	A	43	460	93
	B	–	460	23

[a]Each value presents the average of three determinations, with the exception of the MPN estimates. Each MPN was obtained from one sample.

clostridial agar (Oxoid CM 151) plates before and after heating the cultures at 80 C for 10 min. The plates were incubated anaerobically (atmosphere: 92% H_2; 8% CO_2) at 25 C for 72 h. The MPN of anaerobic and facultative anaerobic spore-forming as well as nonspore-forming bacteria were estimated in the sample with the aid of an MPN table.

Results and discussion — Trimethylamine values greater than 7.0 (mg %N) suggest reject quality cod fillets (Hoogland 1958). The same level of quality is expressed by a taste panel score of 4.5. Examination of Table 1 shows that fillets cut from cod stored on ice for 4 days were unacceptable after 7 days storage, regardless of packaging method. In fact, the storage life of these cod fillets was judged graphycally to be roughly 6½ days (Fig. 1).

Total bacterial and lactobacillus counts (Table 2) were lower in the fillets held in plastic boxes. Large variation normally occurs in total counts due to uneven initial contamination as well as to differences in the weight-to-surface ratios of individual fillets. Hence, the observed differences in total bacteria and lactobacillus counts could be either the result of different experimental treatments or of the variation of the natural bacterial population of the fillets. The yeast and mold counts and the MPN estimates of anaerobic and facultative anaerobic spore and nonspore-forming bacteria did not increase in any of the treatments during the storage period. Thus, there was no evidence to suggest that the storage life or proliferation of various types of bacteria and mold would differ on chilled fillets packaged in wooden as compared to heat-sealed plastic boxes. Ohye and Scott (1957) have shown that some strains of *Clostridium botulinum* Type E organisms can multiply at temperatures as low as 5.0 C (41 F). They showed that the germination of spores at this temperature required an incubation time of 3–4 wk; production of a detectable amount of toxin took 8 wk. The incubation time of 3–4 wk is appreciably longer than the maximum possible storage time of fresh cod fillets. Slocum (1964) has stated that fish fillets inoculated with Type E spores and incubated aerobically and anaerobically, became toxic in 4–5 days at an incubation temperature of 10.0 and 15.6 C (50 and 60 F). The same fish became toxic in 30 days at an incubation temperature of 5.0 C (41 F). The redox conditions necessary for the growth of *C. botulinum* Type E likely existed among the fillets in both wooden boxes and sealed plastic containers. The fact that the spore-forming anaerobic bacteria failed to multiply under the conditions of this experiment suggests that temperature, rather than low oxygen tension, was the limiting factor.

Thus, it is unlikely that in properly chilled fillets ≤5.0 C (≤41 F) packaged in sealed plastic boxes *C. botulinum* would produce toxin during normal storage. The fact that fillets are usually cooked before they are eaten even further decreases the likelihood of botulism, since sufficient cooking is known to inactivate the botulinum toxin.

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Trace Metals in Lake Cayuga Lake Trout (*Salvelinus namaycush*) in Relation to Age

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TONG, S. S. C., W. D. YOUNGS, W. H. GUTENMANN, AND D. J. LISK. 1974. Trace metals in Lake Cayuga lake trout (*Salvelinus namaycush*) in relation to age. J. Fish. Res. Board Can. 31: 238–239.

Chromium concentration increased and molybdenum and tin concentrations decreased in Lake Cayuga lake trout (*Salvelinus namaycush*) from age 1 to 12 yr. The concentration of the rare element rhodium ranged from about 100 to 200 ppb. The concentrations of 33 other trace metals in the fish are reported.

TONG, S. S. C., W. D. YOUNGS ,W. H. GUTENMANN, AND D. J. LISK. 1974. Trace metals in Lake Cayuga lake trout (*Salvelinus namaycush*) in relation to age. J. Fish. Res. Board Can. 31: 238–239.

La teneur en chrome augmente, et celle du molybdène et de l'étain diminuent avec l'âge chez des touladis (*Salvelinus namaycush*) du lac Cayuga âgés de 1 à 12 ans. Un élément rare, le rhodium, s'y trouve à des teneurs allant de 100 à 200 ppb. Nous donnons les teneurs de 33 autres métaux présents à l'état de traces chez ces poissons.

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IN past studies of the concentrations of trace metals in lake trout from Cayuga Lake, only mercury and methyl mercury were found to increase progressively with fish age (Bache et al. 1971). In the work reported here, spark source mass spectrometry (Tong et al. 1969) was used to determine the concentrations of a wide range of trace metals in Cayuga lake trout netted in 1972 and ranging in age from 1 to 12 years.

Procedure — Ages of the fish were accurately known since the lake trout have been stocked there annually as yearlings and are marked as to the date of stocking. Three fish of each age were decapitated and eviscerated and the fins were removed. The remainder was ground and thoroughly mixed. Twenty grams of the mixed tissue was sampled and ashed at 500 C overnight in silica crucibles. The ash was thoroughly mixed and used to form an electrode with graphite by the usual procedure (Tong et al. 1969). Lutecium oxide was added as an internal standard and unit sensitivity was used in the analysis. The metals beryllium, gold, lithium, magnesium, potassium scandium, sodium, and tantalum were not determinable owing to interferences. Most other trace metals not listed in Table 1, if present, were below 0.1 part per billion, the detection limit of the method. The relative deviation of the method was ±25%. Mercury and selenium would have been lost during ashing and are therefore not reported. Indium and callium might have partially vaporized during ashing unless they were present as the less volatile oxides.

Results — Chromium concentration generally increased with trout age while molybdenum and tin decreased (Table 1). Rubidium also decreased after age 7. The correlation between age and concentration was significant for chromium ($r = 0.65$; $P = 0.05$), molybdenum ($r = -0.89$; $P = 0.01$), and tin ($r = -0.76$; $P = 0.01$). No significant correlation between age and concentration was found for the other metals.

The ability of rainbow trout to accumulate chromium in feeding studies has been shown (Knoll and Fromm 1960; Fromm and Stokes 1962). No previous work could be found regarding the mode of absorption of molybdenum, tin, or rubidium by fish. The concentration of rhodium

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TABLE 1. Metal concentrations (ppb fresh weight) in lake trout (*Salvelinus namaycush*) aged 1–12 yr.

Metal	Trout age in years											
	1	2	3	4	5	6	7	8	9	10	11	12
aluminum	200	250	180	220	180	300	230	180	280	140	270	180
antimony	0.46	0.69	0.86	0.52	0.69	0.58	0.46	0.58	0.47	0.61	0.58	0.58
arsenic	80	40	80	72	80	40	80	76	37	79	46	90
barium	17	7	7.3	8.1	4.9	13	7.7	5.6	4.7	9.2	4.1	2.9
boron	0.30	0.25	0.30	0.41	0.63	0.25	0.45	0.25	0.41	0.41	0.25	0.30
cadmium	5.2	6.3	4.1	5.1	5.7	2.8	2.4	3.3	2.9	4.7	3.9	2.1
cesium	4.9	3.4	3.3	4.2	6.7	4.7	6.6	3.3	5.5	7.8	4.1	1.3
chromium	5.2	2.3	3.2	4.1	12	2.8	11	5.2	10	11	32	90
cobalt	70	60	58	81	58	58	58	43	49	62	47	75
copper	36	24	21	21	15	22	24	21	22	20	16	22
gallium	3.1	2.5	2.9	2.3	3.7	1.1	4.3	5.6	3.3	2.8	2.5	1.3
germanium	26	20	16	20	10	26	18	26	16	19	10	10
indium	10	8.5	5.3	7.2	8.5	3.8	8.2	3.8	6.5	8.2	6.9	4.3
iron	190	230	220	200	220	210	270	270	140	310	340	220
lead	22	22	7.4	8.1	7.0	7.7	9.5	7.1	9.5	12	12	4.4
manganese	26	26	26	26	52	26	13	26	17	19	22	27
molybdenum	8.5	8.2	4.5	6.1	5.4	4.5	4.5	3.4	4.5	3.5	2.2	2.8
nickel	8.6	9.0	8.6	9.0	20	8.6	22	22	11	22	7.2	23
niobium	10	8.2	9.2	7.1	7.1	4.5	6.1	6.6	7.5	4.9	9.5	13
palladium	1.3	0.94	0.84	0.92	1.0	0.84	0.84	0.84	0.92	0.10	0.84	0.84
praseodymium	11	11	5.7	7.5	5.3	11	11	11	8.2	6.2	5.3	5.7
rhodium	210	130	210	170	180	210	220	130	160	200	110	140
rubidium	670	670	1400	700	670	1400	1400	400	600	520	58	37
ruthenium	1.3	3.2	1.3	2.2	3.3	1.7	1.6	1.6	1.2	1.2	1.0	1.1
silicon	4200	4400	1100	2200	4200	2100	1100	2200	2900	3100	4500	2200
silver	0.68	0.68	0.68	0.68	0.68	0.68	0.48	0.48	0.58	0.58	0.58	0.48
strontium	120	110	130	140	130	130	150	130	170	110	110	120
tellurium	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9
terbium	0.13	0.25	0.13	0.13	0.13	0.25	0.22	0.13	0.25	0.22	0.13	0.13
thulmium	0.14	0.19	0.32	0.21	0.14	0.14	0.14	0.14	0.23	0.19	0.40	0.14
tin	150	210	210	110	230	92	96	36	96	67	77	44
titanium	24	34	40	31	29	31	37	27	22	20	31	14
tungsten	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
vanadium	2.3	1.7	1.2	2.0	2.3	1.1	2.3	2.3	1.9	1.8	3.4	1.9
yttrium	0.87	0.94	1.8	0.94	1.4	0.50	1.3	0.95	0.88	0.72	0.72	0.95
zinc	290	130	150	220	240	160	35	130	150	370	480	160
zirconium	0.51	0.74	0.74	0.62	0.74	-	0.51	0.37	0.41	0.42	0.30	0.44

appears high in view of the general rarity of the element. No toxic effects of rhodium have been reported from experiments with animals or humans (Browning 1969). In conclusion the concentrations of most metals in lake trout are apparently not related to the age of the fish. The trace concentrations of the metals reported here do not appear to constitute any hazard from a toxicologic standpoint.

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Additional Publications

The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être éxécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter $\overline{\text{O}}$ and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

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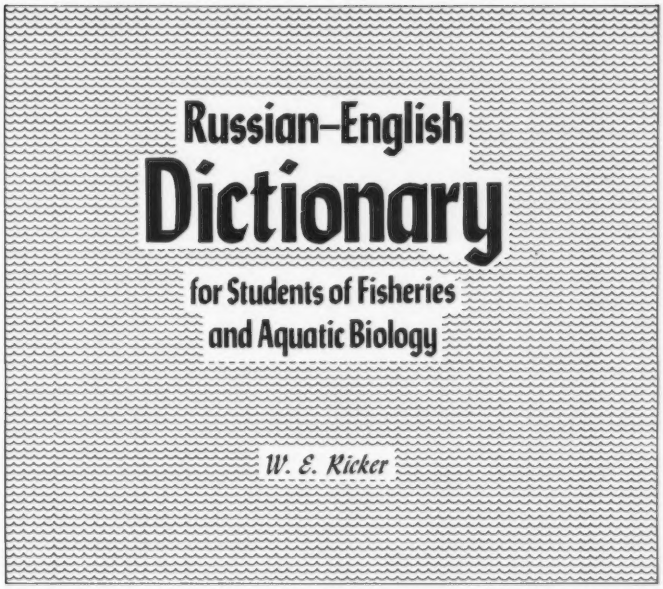
Bulletin of the Fisheries Research Board of Canada



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